Saskatchewan
Environment and
Public Safety

Water Quality
Branch

Application of Aquatic Toxicity Tests to Selected Effluents in Saskatchewan

Recycled Paper

APPLICATION OF AQUATIC TOXICITY TESTS TO SELECTED EFFLUENTS IN SASKATCHEWAN - STUDY RESULTS (1990)

Water Quality Branch
Saskatchewan Environment and Public Safety
WQ 141

November, 1991

TABLE OF CONTENTS

	Page
FOREWORD	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
SUMMARY	vi
RECOMMENDATIONS	viii
1.0 INTRODUCTION	1
1.1 Background	1
1.2 Scope and Objectives	2
2.0 MONITORING PROGRAM	3
2.1 Sampling Locations	3
2.2 Sampling Schedule	7
2.3 Sampling Procedures	7
2.4 Analytical Protocols	10
2.4.1 Rainbow Trout 96-Hour Acute Lethality Test	11
2.4.2 *Daphnia magna* 48-Hour Acute Lethality Test	12
2.4.3 Microtox	12
2.4.4 Nematode (*Panagrellus redivivus*) Survival, Growth, Maturation	13
3.0 RESULTS AND DISCUSSION	15
3.1 Effluent Toxicity Assessment	15
3.1.1 Control Monitoring Sites	15
3.1.2 Industrial Effluents	21
3.1.2.1 Akzo Chemicals	21
3.1.2.2 Saskatoon Chemicals	25
3.1.2.3 Weyerhaeuser Pulp Mill	28
3.1.2.4 8th Avenue East Storm Sewer	31
3.1.2.5 Boundary Flyash Effluent	34

TABLE OF CONTENTS (continued)

		Page
3.1.3	Municipal Sewage Effluents	36
3.1.3.1	City of Saskatoon Effluent	37
3.1.3.2	City of Prince Albert Effluent	42
3.1.3.3	City of Regina Effluent	46
3.1.3.4	Wascana Creek	49
3.2	Analytical Test Assessment	51
3.2.1	Reactions to Control Samples	52
3.2.2	Reactions to Effluent Samples	55

ACKNOWLEDGEMENTS

LIST OF APPENDICES

FOREWORD

In 1990 the Water Quality Branch undertook a review of the need for implementation of toxicity monitoring of effluents and surface waters in Saskatchewan. A pilot study design was prepared to provide the work plan for obtaining some baseline acute toxicity data respecting major effluent discharges and to assess the application of selected bioassays to various types of effluent. The following presentation reports on the findings of this pilot study and provides recommendations for future applications and assessments of acute toxicity test protocols as a water quality monitoring tool.

LIST OF TABLES

		Page
Table 2.1	Toxicity Sampling Locations	5
Table 2.2	1990 Toxicity Sampling Schedule	8
Table 3.1	Summary of Toxic Reactions Detected in Control Samples	53
Table 3.2	Summary of Toxic Reactions Detected in Effluent Samples	57

LIST OF FIGURES

		Page
Figure 2.1	Map Showing Locations of Toxicity Monitoring Sites 1990	4
Figure 3.1	Toxicity Results - Red Deer River (Control Site)	16
Figure 3.2	Toxicity Results - South Saskatchewan River at Outlook (Control Site)	18
Figure 3.3	Toxicity Results - Akzo Chemicals	22
Figure 3.4	Toxicity Results - Saskatoon Chemicals	26
Figure 3.5	Toxicity Results - Weyerhaeuser Pulp Mill	29
Figure 3.6	Toxicity Results - 8th Avenue East Storm Sewer	32
Figure 3.7	Toxicity Results - Boundary Flyash Effluent	35
Figure 3.8	Toxicity Results - City of Saskatoon Sewage Effluent	38
Figure 3.9	Toxicity Results - City of Prince Albert Sewage Effluent	43
Figure 3.10	Toxicity Results - City of Regina Sewage Effluent	47
Figure 3.11	Toxicity Results - Wascana Creek	50

SUMMARY

A pilot study was undertaken in 1990 by the Water Quality Branch to assess the applicability of a battery of four aquatic toxicity test protocols as procedures for evaluating the acute toxicity of selected effluents in Saskatchewan. The study also was designed to use the battery of tests to identify acutely toxic municipal and industrial effluents.

Four tests (Section 2.4) were selected for inclusion in the study. These included acute toxicity tests involving rainbow trout (96 h static) Daphnia magna (48 h static), Microtox and nematode (Panagrellus redivivus) survival, growth and maturation.

A total of 37 water and effluent samples were collected (Table 2.1) by the Branch and analyzed at the contractor's laboratory in Winnipeg. Six quality control samples from clean rivers in Saskatchewan were analyzed as presumably non-acutely toxic surface waters. Sixteen industrial effluent samples from five locations and 15 municipal sewage effluent samples from three major city (e.g. Saskatoon, Prince Albert, Regina) sewage treatment facilities were collected and analyzed between July 21 and October 13, 1990.

Four of the five industrial effluents and two of the three municipal sewage effluents were found to be very acutely toxic in one or more of the samples tested (Appendix 3). Chlorinated primary treated sewage effluents generally yielded higher levels of acute toxicity than did non-chlorinated or de-chlorinated (sodium thiosulphate-treated) primary effluents. In one municipal effluent (Figure 3.10) dechlorination of the effluent fully mitigated acute toxicity to rainbow trout and nematodes suggesting toxicity to these organisms in this high quality tertiary-treated effluent may have been largely due to chlorine residual. Thiosulphate treatment of primary treated effluent (Figure 3.8) only partially reduced toxicity to trout and nematodes and may have increased toxicity to Daphnia. These results for primary treated effluents suggest acute toxicity may be due to other factors than chlorine residual alone.

In the pulp mill effluent acute toxicity was observed on one occasion in rainbow trout (LC_{50} at 42%), Microtox and in nematodes. Daphnia, a proposed regulatory test protocol for mill effluents, was not affected by the mill effluent.

Comparisons were made between the four test protocols regarding test sensitivity to detect toxicity in effluents. On the basis of detection of acute toxicity (e.g. LC_{50} at a concentration of 95% or less) the sensitivity of the four tests were ranked with rainbow trout showing acute toxicity in 18 of 29 effluent samples followed by nematodes (14 samples), Microtox (13 samples) and Daphnia magna (11 samples). However, the nematode tests were carried out at effluent concentrations of 10% or less and hence the response of this test for detection of acute toxicity was likely underestimated. In these same effluent samples, however, significant chronic toxicity in nematodes (inhibition of growth/maturation) was detected in nearly all samples.

Recommendations for further toxicity monitoring of effluents were prepared. These recommendations address:

- the use of a battery of tests for future toxicity evaluation
- the need for further protocol evaluation
- the need for effluent retention/treatment
- the need for concurrent chemical monitoring.

RECOMMENDATIONS

The following recommendations are made with respect to future toxicity monitoring of effluents in Saskatchewan.

1. It is recommended that the battery of tests approach to monitoring be continued to increase the likelihood of detecting acute toxicity in effluents. Additional tests should be considered for inclusion in the battery to provide greater assurance that absence of acute toxicity has not been due to lack of application of sensitive test systems. One algae and one seed germination test are recommended.

2. The four tests utilized provided periodic detections of significant acute toxicity associated with municipal and industrial effluents. Because of the high cost of such analyses industries especially should be required to provide effluent retention systems or other means of reducing the extremes of chemical/physical quality of their effluents. Such retention systems should be an integral part of any wastewater treatment system involving discharges to surface waterbodies.

   Future toxicity monitoring should be accompanied by general chemical monitoring to provide basic water quality information (e.g. pH, salts) about the effluent at the time of toxicity sampling. Unless such is provided costly toxicity (and chemical) monitoring will continue to be a "hit-or-miss" process.

3. Future toxicity monitoring should be carried out at industries and municipal sewage systems to determine which of the individual test protocols provides the most sensitive indication of acute (and/or chronic) toxicity of the effluent(s).

   Emphasis should be on eliminating the need for monitoring with test protocols which may be insensitive and therefore inappropriate for monitoring purposes.

4. Much uncertainty remains about the significance of individual acute toxicity test results. It is recommended that the permit requirement for toxicity testing of effluents be initially limited to those industries/municipalities that discharge relatively large quantities of effluent to surface waters.

5. The Department should undertake split sample analyses with those industries currently conducting toxicity monitoring in order to assess the comparability of results obtained from the respective analytical laboratories.

1.0 INTRODUCTION

1.1 Background

In Saskatchewan numerous municipal and industrial wastewater treatment systems discharge their final effluents to receiving waterbodies. The province has established general effluent discharge objectives, mixing zone guidelines and surface water quality objectives (Saskatchewan Environment and Public Safety, 1988) to protect surface waters from unacceptable impacts due to discharges. These objectives and guidelines include the requirement for wastewater treatment facilities to produce effluent of a quality that is not acutely toxic to aquatic life. Information respecting the acute toxicological characteristics of effluents in Saskatchewan, however, was lacking. It was therefore recognized that toxicity assessment was required and a plan was initiated to undertake a pilot study involving toxicological monitoring of selected effluents in Saskatchewan. The intent of the study was to help identify major toxic effluent sources and to assess the performance of selected bioassay procedures for toxicity assessment of effluents.

In spring of 1990 the Water Quality Branch completed a literature review and study plan regarding the selection of aquatic toxicity tests to be assessed as monitoring tools in the evaluation of acute toxicity of effluents (Lauten,

1990, unpublished, File F10-2-2, April, 1990). The study plan included the use of a battery of four toxicity test protocols to be applied to evaluate acute toxicity in several major industrial and municipal effluents which are discharged, after treatment, to surface waterbodies. This report describes the toxicity monitoring study carried out in 1990.

1.2 Scope and Objectives

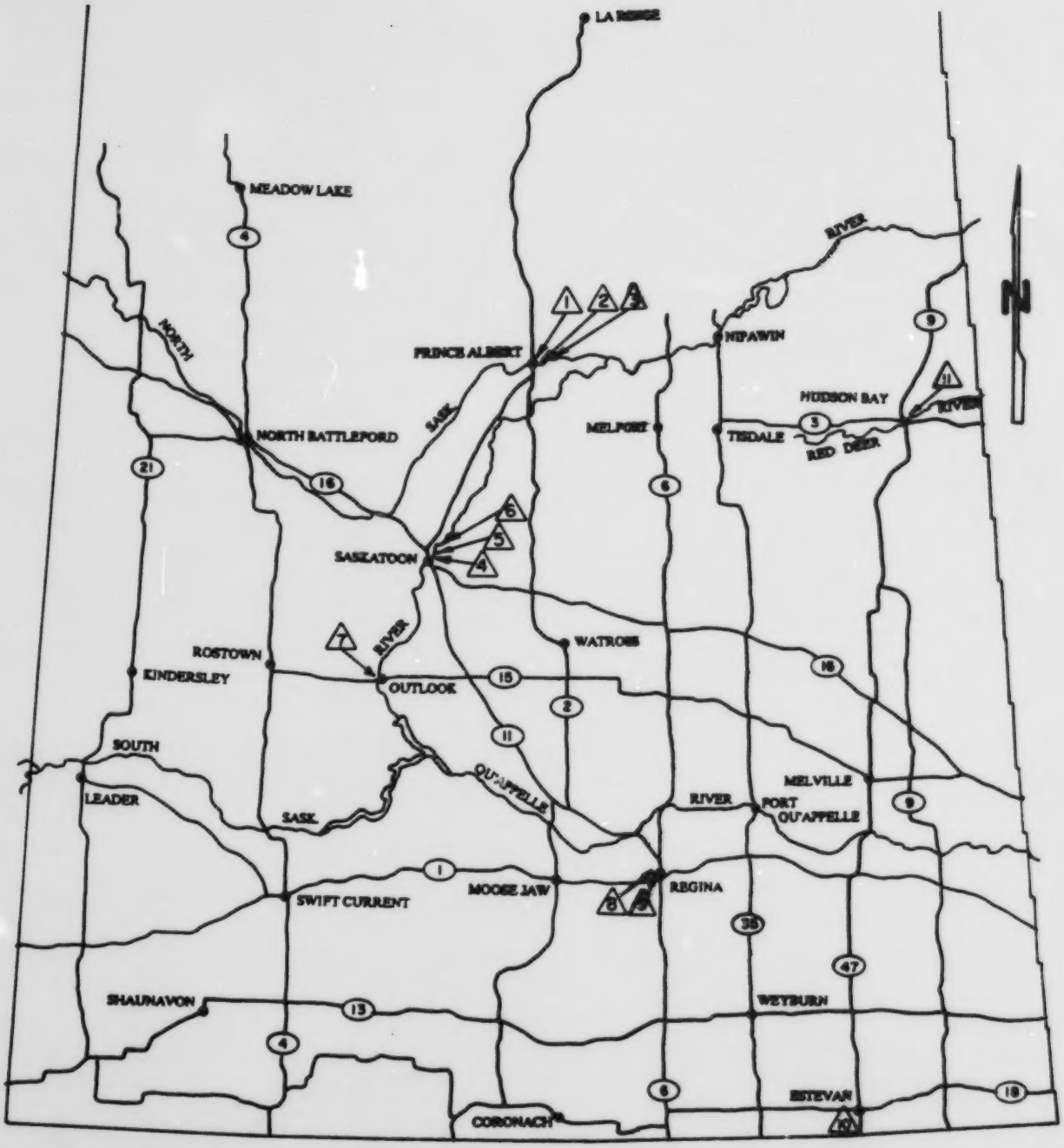
The purpose of the study was to select a number of bioassay protocols which could be used in a pilot study to obtain information about the acute toxicity of various effluent discharges to surface waters. Also, the study was designed to apply test protocols to identify which of the selected effluents were acutely toxic and which should therefore be more closely monitored and regulated. The study was also carried out to provide Water Quality Branch staff with experience in toxicity monitoring and interpreting test protocol results and to obtain information needed to make recommendations respecting regulatory toxicity monitoring. It was anticipated that valuable information about the logistics of toxicity monitoring and valuable experience in sampling effluents of diverse quality characteristics would also be obtained.

2.0 MONITORING PROGRAM

The study plan prepared in April, 1990 (Lauten, 1990, File F10-2-2, unpublished) served as the initial guide in study site and analytical protocol selection, as well as serving as the general schedule respecting timing of sample collections. The study plan was also used to prepare Terms of Reference which were used to solicit tenders for laboratory services required for carrying out the acute toxicity analyses. Following the tendering process an analytical laboratory was selected and awarded a contract to conduct acute toxicity analyses of samples collected and submitted by Water Quality Branch staff.

2.1 Sampling Locations

Eleven effluent and surface water locations were selected as monitoring sites. Three municipal sewage effluents, five industrial effluents and three surface waterbodies comprised the list of monitoring locations. These sites are each described in Table 2.1. The Department's ESQUADAT station codes for each location are also provided in Table 2.1. Their locations in Saskatchewan are shown in Figure 2.1.



LEGEND

△ Toxicity Monitoring Site

1. Prince Albert Sewage Effluent
2. 8th Ave. East Storm Sewer
3. Weyerhaeuser Pulp Mill Effluent
4. Saskatoon Sewage Effluent
5. Akzo Chemical Effluent
6. Saskatoon Chemicals Effluent
7. South Saskatchewan River at Highway No. 15
8. Regina Sewage Effluent
9. Wascana Creek Above Qu'appelle River
10. Boundary Generating Station Flyash Effluent
11. Red Deer River at Highway No. 3

Figure 2.1 Map Showing Location of Toxicity Monitoring Sites, 1990.

Table 2.1

TOXICITY SAMPLING LOCATIONS

1. Red Deer River above Highway 3 bridge sampled 10 m from left bank. The site is located about 12 km downstream of the Town of Hudson Bay in northeastern Saskatchewan. One sample was obtained from this location on July 22, 1990. The site was considered as a possible control station.

   ESQUADAT Station Code 00SK12100003

2. South Saskatchewan River near Outlook. This site was selected as a control station as the river at this location below Lake Diefenbaker is not subject to effluent discharges. Sampling was carried out at Highway 15 off left bank.

   ESQUADAT Station Code 00SK06220002

3. Akzo Chemicals (Saskatoon) final effluent. This industry produces glycerine, amines, amides and other organics. The chemical and physical properties of the effluent are highly variable with extremes of pH and high levels of ammonia being notable features. Discharge is to the South Saskatchewan River. A recent report characterizes the chemical effluent quality (SEPS, 1991).

   ESQUADAT Station Code 63SK06230001

4. Saskatoon Chemicals (Saskatoon) final effluent. This industry produces bleaching agents and additives required by the pulp and paper industry.

   The effluent quality is highly variable with respect to pH. Discharge is to the South Saskatchewan River. A recent report characterizes the effluent chemical quality (SEPS, 1991).

   ESQUADAT Station Code 63SK06230009

5. Weyerhaeuser Pulp Mill (Near Prince Albert) final effluent. The effluent was sampled at the outlet weir of the secondary aeration pond. Discharge ranges from 60,000 to 90,000 m^3/day with the receiving water being the North Saskatchewan River. The water quality characteristics of the mill effluent were characterized in detail in a previous report (Saskatchewan Environment, 1984b).

   ESQUADAT Station Code 63SK07200001

6. 8th Avenue East (Prince Albert) storm sewer outfall. The storm sewer carries contaminated groundwater from at least two former wood preserving plants located within the City of Prince Albert. Creosote, pentachlorophenol, polyaromatic hydrocarbons and dioxins/furans occur in the discharge. Various booms (hay bales, absorbent mats) are located at the discharge site to retain creosote and associated oils. Discharge is to the North Saskatchewan River as a surface shoreline outfall.

   ESQUADAT Station Code 21SK07200005

7. Boundary Thermo-Electric Generating Station flyash effluent. Flyash ponds were used to settle ash remaining after combustion of coal at the thermoelectric generating station operated by the Saskatchewan Power Corporation. Discharge from the ponds was into the power station's cooling water effluent channel which in turn flows into Boundary Reservoir. Recently, discharge from the flyash ponds was discontinued. High salt and boron levels characterized the flyash effluent. Water quality characteristics of Boundary Reservoir were reviewed in a previous report (Saskatchewan Environment, 1983). Sampling was carried out on two occasions at the outlet of flyash pond No. 1.

   ESQUADAT Station Code 66SK01100003

8. City of Saskatoon Municipal Sewage Final Effluent. The discharge was sampled at the outlet weir of the facility on 3 occasions - July 20, August 17 and September 7, 1990. On the two latter occasions duplicate samples were obtained and one duplicate treated with sodium thiosulphate (2.5 g/20L) to neutralize chlorine residual. The effluent is discharged to the South Saskatchewan River following advanced primary treatment and disinfection with chlorine.

   ESQUADAT Station Code 76SK06230001

9. City of Prince Albert Sewage Final Effluent. Sewage at the Prince Albert treatment facility undergoes primary treatment and disinfection using chlorine prior to discharge to the North Saskatchewan River. The effluent quality characteristics were previously reviewed and reported (Saskatchewan Environment, 1984b). Sampling was carried out at two locations to obtain non-chlorinated and chlorinated effluent.

   ESQUADAT Station Code 73SK07200002

Table 2.1 (continued)

10. City of Regina Sewage Effluent. Sampling is carried out at the mouth of the discharge pipe as it enters Wascana Creek. The treatment facility uses large retention aerated lagoons and the effluent is tertiary treated and disinfected with chlorine (during open water seasons) prior to discharge to Wascana Creek. The effluent is generally of high quality but ammonia levels have raised some concerns about aquatic life in the receiving water. The effluent quality characteristics have been reviewed in previous reports (Tones, 1981; Saskatchewan Environment, 1984a). Duplicate samples were obtained on two occasions with one duplicate treated with thiosulphate to neutralize residual chlorine.

    ESQUADAT Station Code 75SK05130001

11. Wascana Creek just above Qu'Appelle River. The creek is the receiving waterbody for tertiary treated, disinfected effluent from the City of Regina sewage treatment facility. Sampling was carried out at the mouth of the creek to assess possible toxicity related to effluent discharge upstream.

    ESQUADAT Station Code 00SK05130008

2.2 Sampling Schedule

Sampling was conducted at the eleven locations described in Section 2.1 at the frequency and dates shown in Table 2.2.

2.3 Sampling Procedures

To provide sufficient sample volume to carry out the four bioassay procedures (Section 2.4) 80 L samples were obtained. Four - 20 L red plastic jerry cans were used as sample containers. In most instances effluent samples were obtained using a 4 L clean plastic or stainless container

Table 2.2

1990 TOXICITY SAMPLING SCHEDULE

Sampling Site	Effluent or Sample Type	Sampling Dates 1990	Number of Samples
1. Red Deer River, Highway 3	Control	July 22	1
2. South Saskatchewan River, Outlook	Control	August 17, 23, 30 Sept. 6, 21	5
3. Akzo Chemicals	Industrial	August 17 Sept. 7, 14	3
4. Saskatoon Chemicals	Industrial	August 17 Sept. 7, 14	3
5. Weyerhaeuser Pulp Mill	Industrial	July 21, August 29 Sept. 14, October 13	4
6. 8th Avenue East Storm Sewer	Industrial	July 21, August 29 Sept. 14, October 5	4
7. Boundary Flyash	Industrial	August 30 Sept. 22	2
8. City of Saskatoon	Municipal	July 20, August 17 Sept. 17	5
9. City of Prince Albert	Municipal	October 5, 13	4
10. City of Regina	Municipal	August 23 Sept. 22	4
11. Wascana Creek	Receiving Water	August 23 Sept. 21	2
			37

as a bailer to fill each of the four - 20 L sample containers. In some locations where it was safe to do so and physically possible to submerge the 20 L jerry cans or direct effluent flow into them, the jerry cans were filled directly with water or effluent. All jerry cans were rinsed thoroughly with sample water/effluent just prior to filling with the sample liquid.

After sampling, the jerry can samples were shipped by bus from Regina or other major centres to the analytical

laboratory in Winnipeg. In general, no more than 3 days elapsed between the time of sampling and the start of laboratory analyses. Following completion of laboratory analyses, the remaining sample material was discarded into the municipal sanitary sewage system, the container rinsed with tap water, and the containers returned to the department by bus transit. Most containers were cleaned for re-use with the exception of those used to sample the 8th Avenue East storm sewer in Prince Albert. These storm sewer sample containers were discarded due to the large amounts of creosote and oily material that would adhere to the interior of the jerry cans.

In all instances, the sample containers were labelled with code numbers and the receiving laboratory only informed of the types of potential hazards to health that might be associated with each sample (e.g. bacteriological, chemical hazards). For samples with specific characteristics which might impact upon the test results the laboratory was forewarned. Such characteristics included high biochemical oxygen demand, extremes of pH, and high salt concentrations.

2.4 Analytical Protocols

Four bioassay protocols were selected for application to effluents in Saskatchewan. The four were selected on the basis of their acceptability as bioassay protocols, their current or past use in Saskatchewan or for effluent analyses, economic and humanitarian considerations and the availability of the test analyses at an accessible laboratory (see Lauten, 1990, unpublished). The four protocols included test organisms from several different trophic levels including trout, Daphnia, bacteria, and nematodes. Tests with algae/aquatic plants were not included in this pilot study for economic reasons.

All toxicity analyses were conducted by staff of Bioquest International Incorporated of Winnipeg, Manitoba, under the direction of Dr. Martin Samoiloff. This bioassay laboratory was selected to be the contractor to provide analytical services for this pilot study following review of tenders submitted to the department. Economics and technical expertise were the primary deciding factors in selecting this company as the analytical laboratory.

The following sections summarize the analytical protocols of the four bioassays selected for this study. Detailed protocols and supporting references for each bioassay

procedure were provided by the contractor during the tendering process and are attached to this report as Appendix 1.

2.4.1 Rainbow Trout 96-hour Acute Lethality Test

This static test measures the survival of sets of ten three-month old rainbow trout over a 96-hour exposure period. Each set of trout is exposed to a specific concentration of the test sample made up to a 6 L volume in glass aquaria. The relationship between concentration and the number of fish dying during the test period is used to estimate the concentration at which 50 per cent of the fish die in 96 hours (the LC_{50}).

Samples were aerated to maintain dissolved oxygen above 6 ppm. It was recognized that aeration might reduce the net toxicity of the samples (due to greater volatilization, venting, oxidation). Some samples of effluent had high oxygen demands necessitating aeration to prevent fish deaths due to suffocation. Control aquaria consisted of one 6 L volume of dechlorinated, carbon filtered Winnipeg municipal water containing 10 rainbow trout.

No adjustment of pH was attempted with any sample.

2.4.2 Daphnia Magna 48-hour Acute Lethality Test

This test measures the survival of newborn Daphnia magna (water fleas) over a 48 hour test period. Tests are performed using 10 juvenile Daphnia in beakers containing 100 ml of sample dilutions. Dilution water, and water used for control tests consisted of reconstituted hard water derived from the Winnipeg municipal water system.

2.4.3 Microtox

The Microtox test measures the reduction in bioluminescence of a quality-controlled population of marine bacteria during exposure to the tested sample. This reduction is a measure of the toxicity of the sample; the more toxic the material, the greater the reduction of luminescence. Healthy cells emit more light than do cells exposed to toxic materials. In the Microtox test, the light output of a healthy, unexposed population is compared to the light output of cells exposed to a range of concentrations of test material. The range of test concentrations is from 2.8% of the test sample to 45.4% of the test sample. Plotting the amount of light reduction against concentration of test

material allows the estimation of the concentration at which a 50% reduction in light (the EC_{50}) occurs and the concentration at which light production is reduced by 20% (the EC_{20}). Readings are made after 5, 10 and 15 minute exposure to the test material.

This test utilizes commercially prepared test organisms and reagents and because of the short time period required for performing the test it has gained wide application in assessing the toxicity of a wide variety of sample material. It utilizes minute quantities of sample and requires relatively high capital investment for the electronic analyzer used to measure luminescence.

2.4.4 Nematode (Panagrellus redivivus) Survival, Growth, Maturation

The Panagrellus redivivus test determines the survival, growth and maturation of a synchronously growing population of this nematode. The test starts with a population of 100 new born animals (10 in each of 10 small containers) and concludes when a minimum of 50% of a control population reaches maturity, a period of 96 hours. The control population serves as a reference for normal survival, growth and maturation. Effects of these

parameters in the exposed population are expressed as a percentage of these values for the control population. Survival is an indication of effects of the tested material on essential processes. Growth is a measure of the effects of the tested material on the overall physiology of the test animals, while maturation reflects the impact of the test substance on gene-level activities. The effects are summarized by a weighted mean, termed fitness. A fitness of 100 indicates no effect, while decreased values of fitness indicates adverse biological effects. Fitness values greater than 100 indicate the presence of nutrients or growth promoting materials. Emphasis in this report, however, is directed towards the results concerning survival, growth and maturation.

Tests are run at 10% and 1% dilutions of tested sample or other desired dilutions. Food supplemented phosphate buffer (M-Y) was used as dilution medium.

3.0 RESULTS AND DISCUSSION

The results of toxicity analyses are presented in this chapter to assess the acute toxicity of various effluents examined (e.g. Section 2.1) and to evaluate the 4 bioassay procedures as tools for toxicity monitoring of effluent discharges in Saskatchewan.

3.1 Effluent Toxicity Assessment

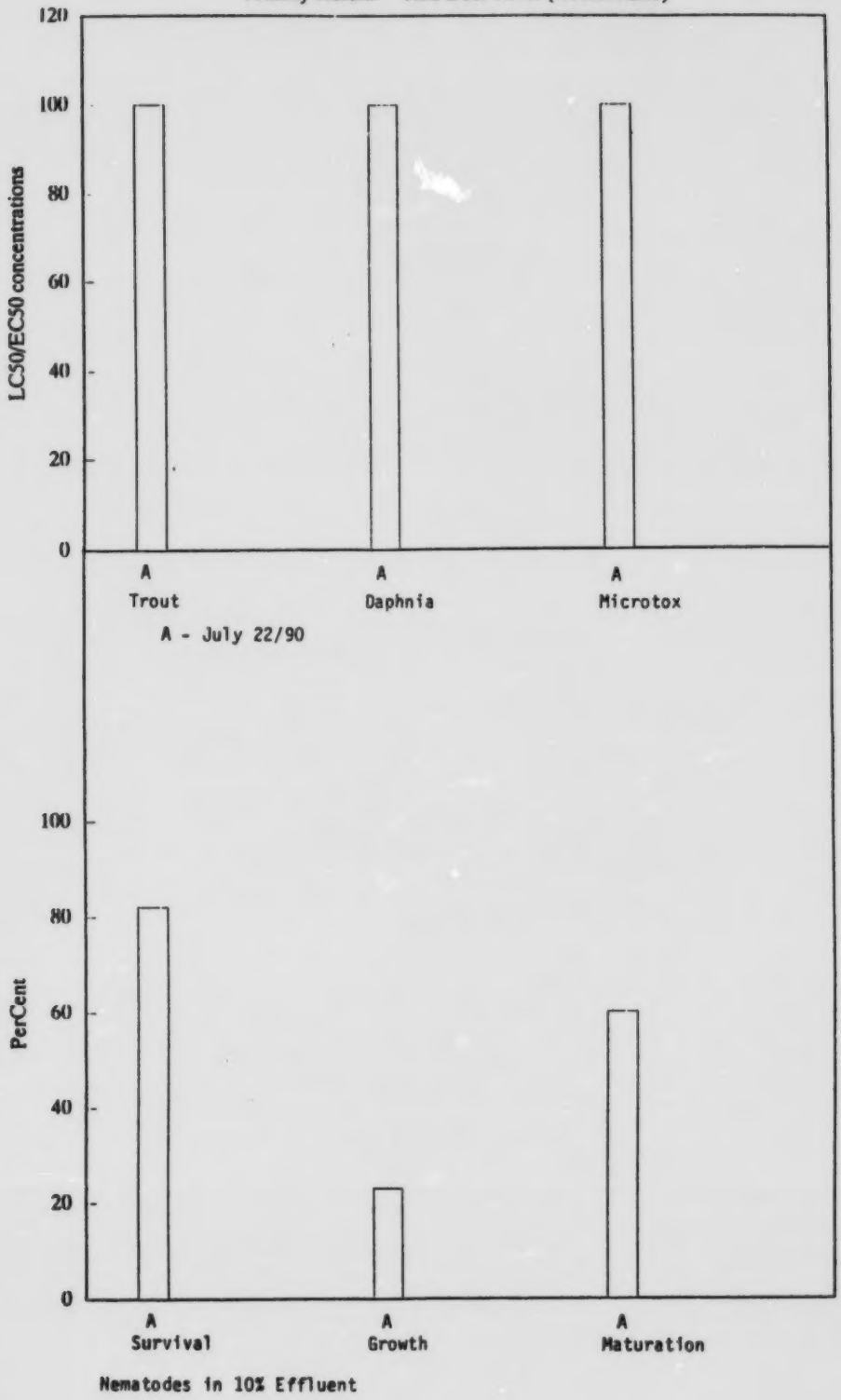
3.1.1 Control Monitoring Sites

Two control monitoring sites were utilized in the present study to assess the reaction of the 4 bioassay procedures when applied to surface waters where no known or significant effluent discharge was occurring and where no source of toxicity was suspected. These two locations were the Red Deer River at Highway 3 bridge and the South Saskatchewan river near Outlook (Table 2.2). The Red Deer River site was sampled only on one occasion as logistical concerns (e.g. travel distance) precluded its routine monitoring. The South Saskatchewan River site was selected for regular monitoring since it is sampled on a monthly basis as one of the province's primary water quality monitoring network stations.

The results of analyses for the Red Deer River site are shown in Figure 3.1. No toxicity was indicated

Figure 3.1

Toxicity Results – Red Deer River (Control Site)

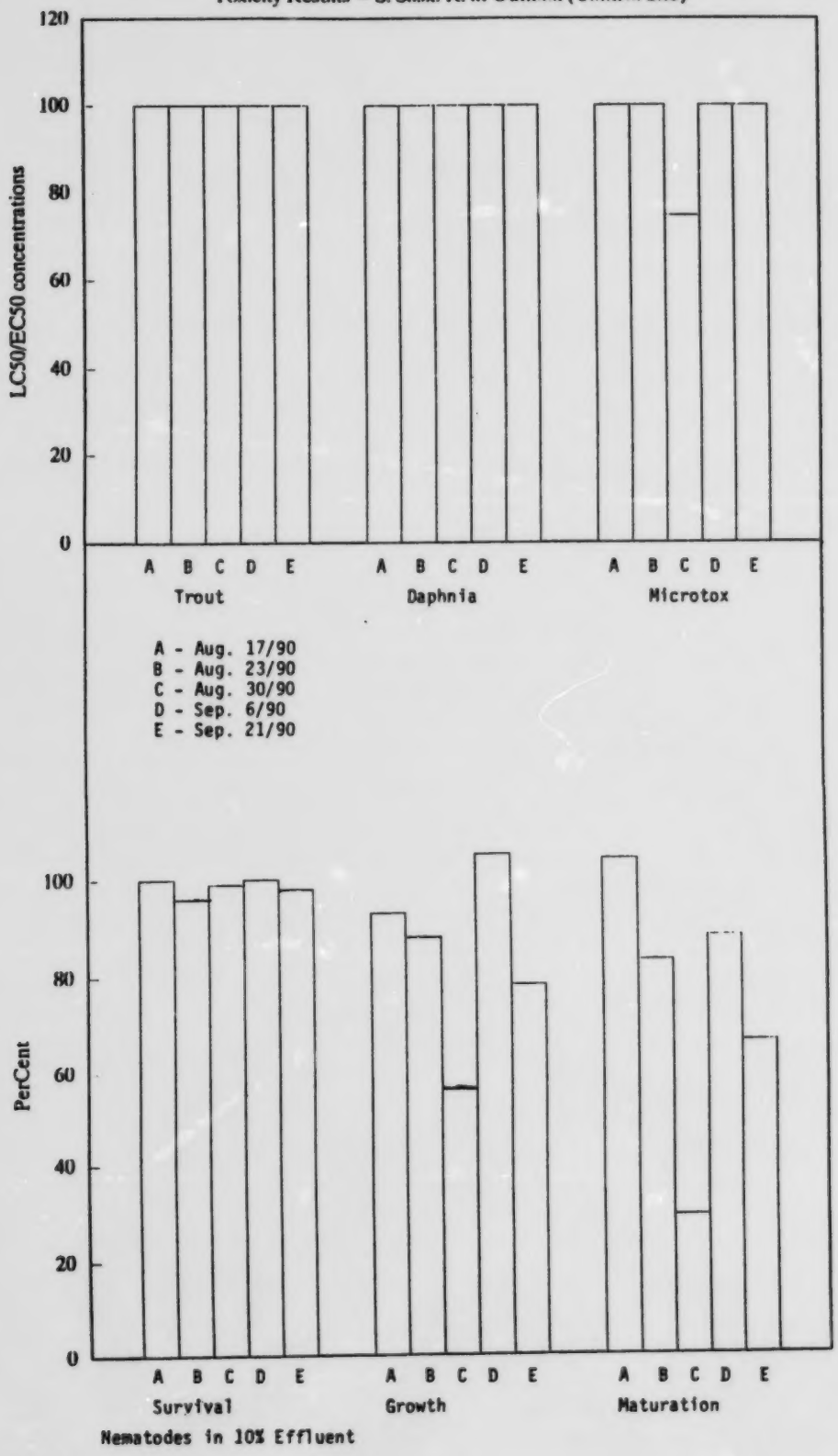


by the rainbow trout, Daphnia or Microtox tests as there were no fatalities of fish or Daphnia, and no inhibition of luminescence in bacteria with the raw water (100% concentration of sample) sample. Moderate toxicity was indicated by the results of the nematode test, however. Eighty-two per cent of worms survived exposure to a 10 per cent dilution of sample water. Growth was observed in only 23 per cent of test animals and only 60 per cent of the worms developed to maturity over the 4 day incubation. The resulting fitness number (62) suggests some moderate toxicity was present as no such toxic response was recorded for control animals in the laboratory.

The results of tests on surface water from the South Saskatchewan River near Outlook are summarized in Figure 3.2. This site was sampled on five occasions between August and September of 1990 (Table 2.2). No toxicity was indicated in any of the five samples by either the rainbow trout or Daphnia bioassays (e.g. all LC_{50} values were greater than 100 per cent). In only one sample, collected August 30, 1990, was toxicity indicated by the Microtox test result. The EC_{50} value for this sample, or the concentration of sample (river

Figure 3.2

Toxicity Results – S. Sask. R. at Outlook (Control Site)



water) which reduced bioluminescence by 50%, was 75 per cent. This suggests a slight toxic response of the test bacteria to the 75% dilution of river water.

In the same (August 30, 1990) sample that elicited the slight toxic response in the Microtox test 99% of the test nematodes survived the 4 day incubation in a 10% dilution but only 56% showed growth, and only 29% reached maturity. The overall fitness value for the nematodes in this sample test was 77. For this same sample prepared as a 1% dilution no significant acute toxicity was observed (e.g. survival 98%) and no significant indication of chronic toxicity was recorded (e.g. growth 94%, maturation 107%, fitness 98%).

Amongst the other four samples nematode survival in the 10% test dilutions ranged from 96 to 100% indicating no significant acute toxicity. For growth and maturation (potential indicators of chronic toxicity) the range of values were 78 to 100% and 66 to 104% respectively. The respective fitness values for these four samples ranged from 88 to 100%. The minima for each measurement occurred in the same samples.

These nematode results are difficult to explain. Relative to the laboratory controls, which were suspended in phosphate buffer (e.g. not exposed to sample water), the 10% river water dilution reduced growth and/or maturation and for one sample at least, this chronic toxicity was mitigated by further dilution of the river water to 1% using phosphate buffer which comprised 100% of the control group liquid medium. This might suggest that the river water might not have been conducive to growth and maturation of the nematodes rather than the results being caused by direct chronic toxicity related to some substance or substances in the river water.

The important consideration respecting the results of the nematode test applied to these control river water samples was that no significant acute toxicity was observed in the 10% dilutions. Further application of this test system should include tests with higher concentrations of test water (receiving waters, effluents) with perhaps phosphate buffer added to adjust the ionic conditions to more closely approximate that of the control culture.

3.1.2 Industrial Effluents

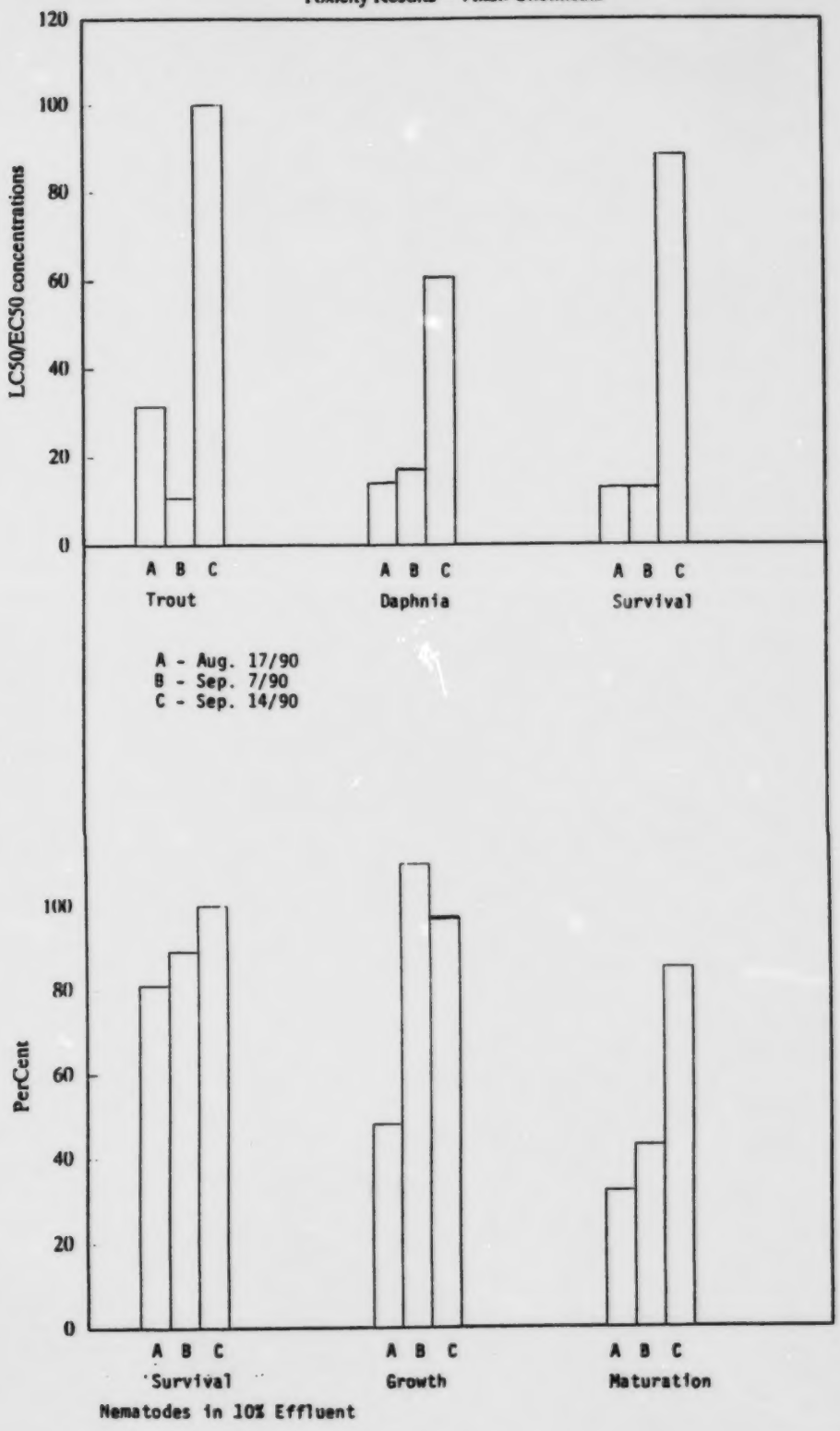
Five industry-related effluents were examined in 1990 (Table 2.1) with a total of 17 samples being analyzed using the four bioassay procedures (Section 2.4).

3.1.2.1 Akzo Chemicals

The results of analyses for three samples from this industry are shown in Figure 3.3. The two samples obtained on August 17, 1990 and September 7, 1990 were very acutely toxic to rainbow trout and Daphnia, and showed a high level of acute toxicity in the Microtox test. For instance, for the August 17, 1990 sample the LC_{50} values for trout and Daphnia were reached at effluent concentrations of 32 and 14% respectively. The EC_{50} value for Microtox occurred at an effluent concentration of only 13% on both these dates. For the September 7, 1990 sample the rainbow trout and Daphnia LC_{50} values occurred at effluent concentrations of 11 and 17%, respectively.

Figure 3.3

Toxicity Results – Akzo Chemicals



For these two samples moderate acute toxicity occurred in nematodes with 81 and 89% of test worms surviving exposure to 10% dilutions of effluent from the August 17 and September 7, 1990 sampling dates. Growth and maturation were reduced to 48 and 32% respectively in the August 17 sample but there was a stimulation of growth in the September 7 sample which occurred, however, with reduced maturation (e.g. 43%).

In the third sample taken September 14, 1990, no rainbow trout died but acute toxicity was observed for Daphnia with an LC_{50} value recorded at 61% effluent concentration. Only minor reduction in light emission was observed in the Microtox test (EC_{50} at 86%) and no significant acute toxicity or suppression of nematode growth or maturation was observed.

Of interest regarding the nematodes were the results for the test of the first two samples conducted at the 1% effluent concentration. For the August 17 sample, the dilution to 1% mitigated all acute toxicity (e.g. survival was 99%) but the percentage of worms that grew and matured in a manner comparable to the control culture remained low at 44 and 26%, respectively. The September 14, 1990 sample, when analyzed at 1% dilution, displayed no acute or chronic toxicity indicating that further effluent dilution permitted normal growth and maturation that had been inhibited at the 10% effluent concentration.

These results for toxicity testing of the Akzo Chemicals effluent showed the effluent to be very acutely toxic in two of the three samples and that chronic toxicity (inhibition of nematode growth/maturation) occurred in one sample even when the effluent was diluted to 1/100th its original strength.

The varied results between sampling dates may relate to high variability of effluent water quality since there is batch processing of various products and because there are several effluent streams within the industry and no significant retention or storage or pooling of effluent prior to discharge.

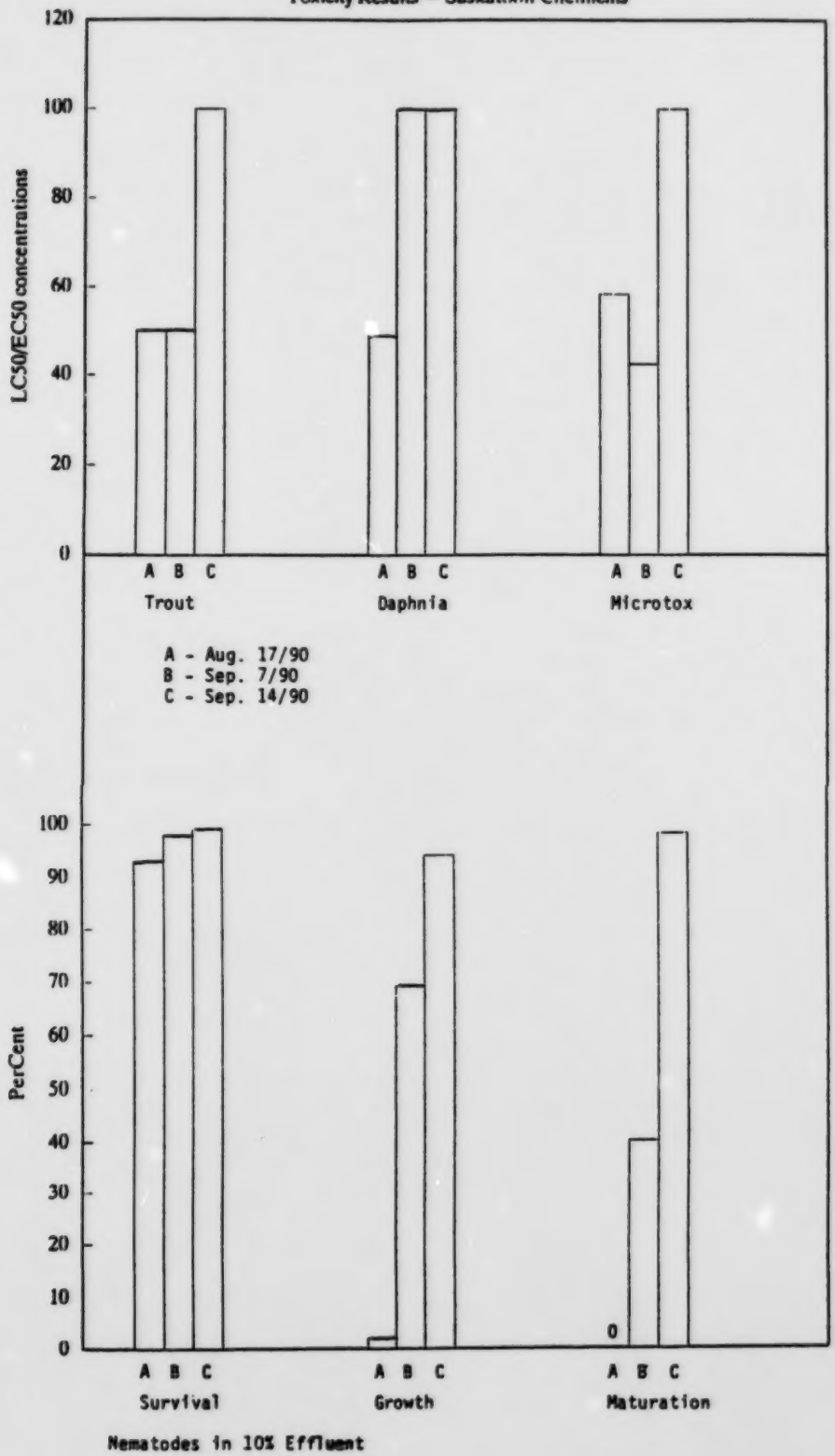
3.1.2.2 Saskatoon Chemicals

Three effluent samples from this industry were collected for analyses (Table 2.1). The results of analyses are summarized in Figure 3.4.

The results of analyses showed the effluent to be acutely toxic to rainbow trout (LC_{50} at 50% concentration) in 2 of the 3 samples tested (August 17 and September 7, 1990). A similar result for Daphnia (LC_{50} at 49%) and Microtox (EC_{50} 59%) was observed for the August 17 sample but no acute toxicity to Daphnia was recorded for the latter two samples. The effluent did not appear to be acutely

Figure 3.4

Toxicity Results – Saskatoon Chemicals



toxic at the 10% test concentration for nematodes as most worms survived the exposure in all three samples. However, only 2% of worms displayed normal growth and none reached maturity after exposure to the 10% dilution of the August 17, 1990 sample. At the 1% dilution of this sample inhibition of growth and maturation was only partially mitigated as only 41% grew and only 32% reached maturity.

The September 7 sample was not acutely toxic at the 10% concentration to nematodes but growth (41%) and maturation (32%) were impaired. For this sample diluted to 1% the only significant impairment to nematodes was with respect to maturation which remained low at 53%. This sample also impaired light emission in bacteria in the Microtox test resulting in a EC_{50} value of 43%.

The September 14, 1990 sample had no acute or significant chronic toxicity response in any of the four bioassays.

The battery of tests used to assess the acute toxicity of this effluent showed it to be acutely toxic in two of the three samples tested. Impairment of growth and maturation of nematodes was also observed in these two acutely toxic samples even though the samples did not significantly reduce nematode survival at the 10% test concentration of effluent.

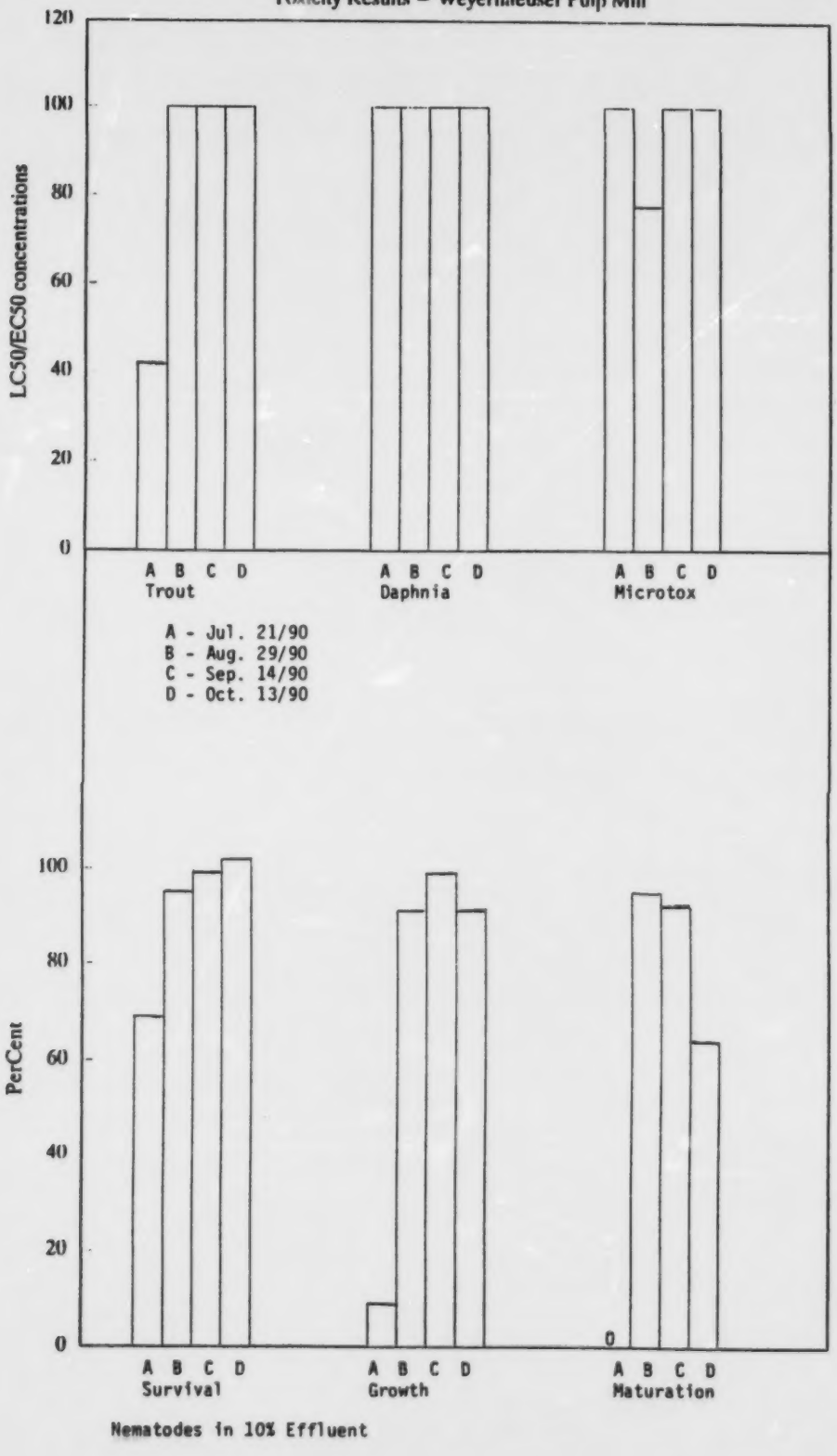
3.1.2.3 Weyerhaeuser Pulp Mill

Four samples of final effluent were collected for toxicity analyses in 1990. The results are summarized in Figure 3.5.

The pulp mill effluent caused relatively little toxic response in the four tests applied to it. None of the four samples were acutely toxic to Daphnia. Only the first sample tested (July 21, 1990) was acutely toxic to rainbow trout and resulted in an LC_{50} value of 42%.

Figure 3.5

Toxicity Results – Weyerhaeuser Pulp Mill



Nematodes in 10% Effluent

The Microtox test showed toxic response only to the August 29, 1990 sample which provided an EC_{50} value at an effluent concentration of about 78%.

Similarly, acute toxicity in nematodes was only observed in the July 21, 1990 sample in which only 42% of worms survived the 4 day exposure to the 10% concentration of effluent. From 95 to 100% of the nematodes survived exposure to the other three samples. The July 21, 1990 sample, however, severely inhibited the growth and maturation of the nematodes in the 10% effluent dilution. Only 9% of test worms showed normal growth but none of the worms reached maturity. The other three samples had only minor inhibitory influences on nematode growth and maturation with the exception of the October 13, 1990 sample in which only 65% of worms matured.

Over 90% of nematodes in these three samples grew normally, relative to the control culture, however. In these samples diluted to 1% concentration no acute toxicity or inhibition of growth or maturation was observed.

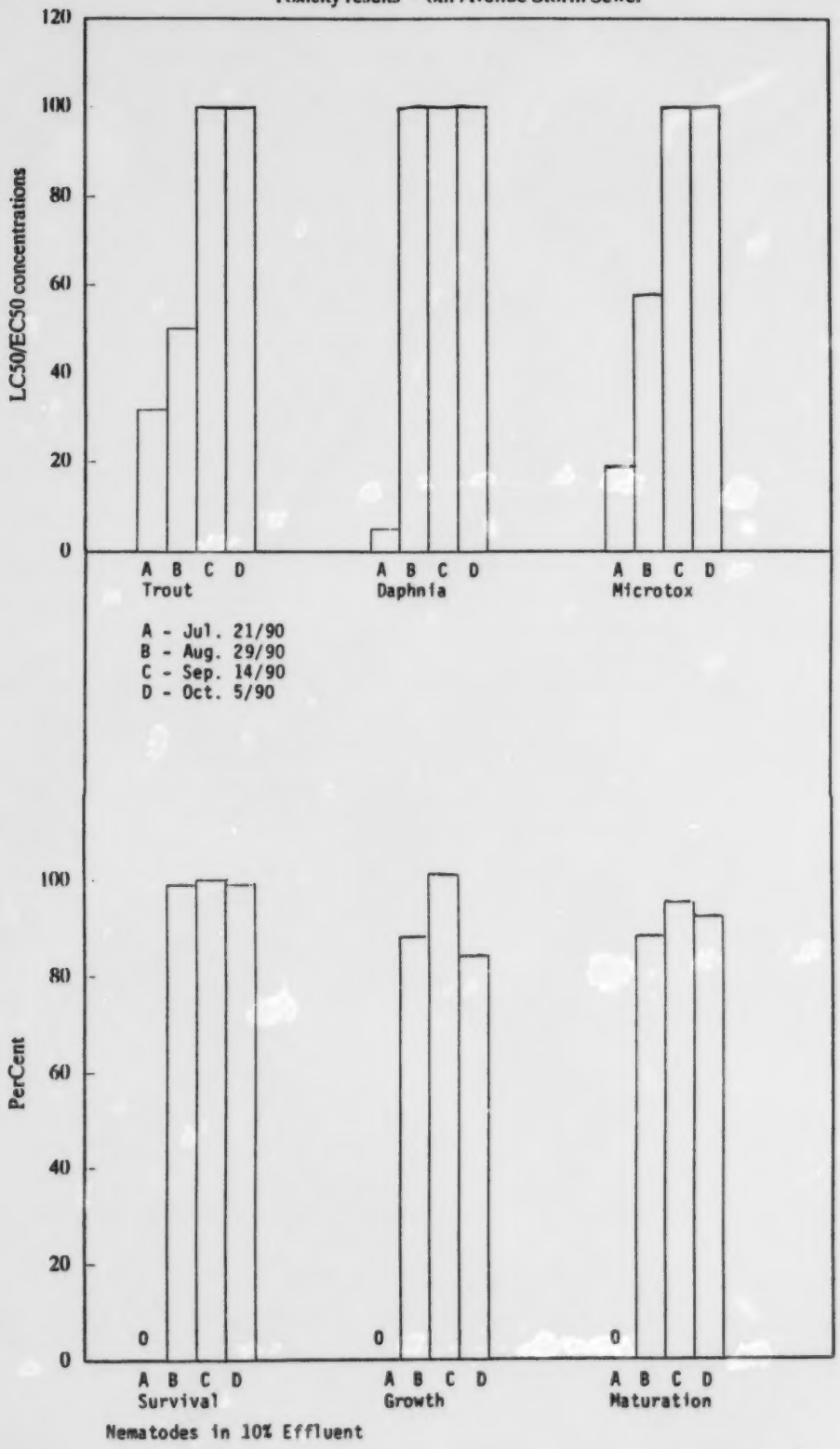
These results suggest that acute toxicity might be rarely observed in the pulp mill effluent using the four test procedures. Daphnia was unaffected by any of the four samples suggesting this test organism may be relatively insensitive to the mill effluent. Similarly, Microtox displayed moderate toxicity response in only one of the four samples. Moderate lethality to nematodes occurred in only one sample (July 21, 1990) but in that sample growth and maturation were severely inhibited. It was also this sample which caused lethality in one-half the test rainbow trout at an effluent concentration of 42%.

3.1.2.4 8th Avenue East Storm Sewer

This sampling source was perhaps the most irregular with respect to predictability of discharge or discharge quality. Four samples were collected from this site for analyses (Table 2.1). The results are summarized in Figure 3.6.

Figure 3.6

Toxicity results – 8th Avenue Storm Sewer



The July 21, 1990 sample was shown to be highly acutely toxic by all four of the bioassays. Rainbow trout were very sensitive to this sample with the LC_{50} occurring at an effluent concentration of 32%. For Daphnia the LC_{50} occurred at 5%. The EC_{50} for the Microtox test was at 19%. The effluent, diluted to 10%, killed all nematodes (e.g. survival was 0%).

The August 29, 1990 sample was also acutely toxic to rainbow trout (LC_{50} at 50%) but was not acutely toxic to Daphnia. The Microtox EC_{50} for this sample was 58%. No acute toxicity occurred in nematodes at a 10% dilution of this effluent sample and growth and maturation were only marginally reduced (both values 88%).

The remaining two samples caused no acute toxicity in any of the four bioassays. Only marginal inhibition of growth and maturation of nematodes was recorded for these two samples at the 10% dilution whereas dilution to 1% concentration of effluent virtually eliminated observable inhibition of nematode growth/maturation.

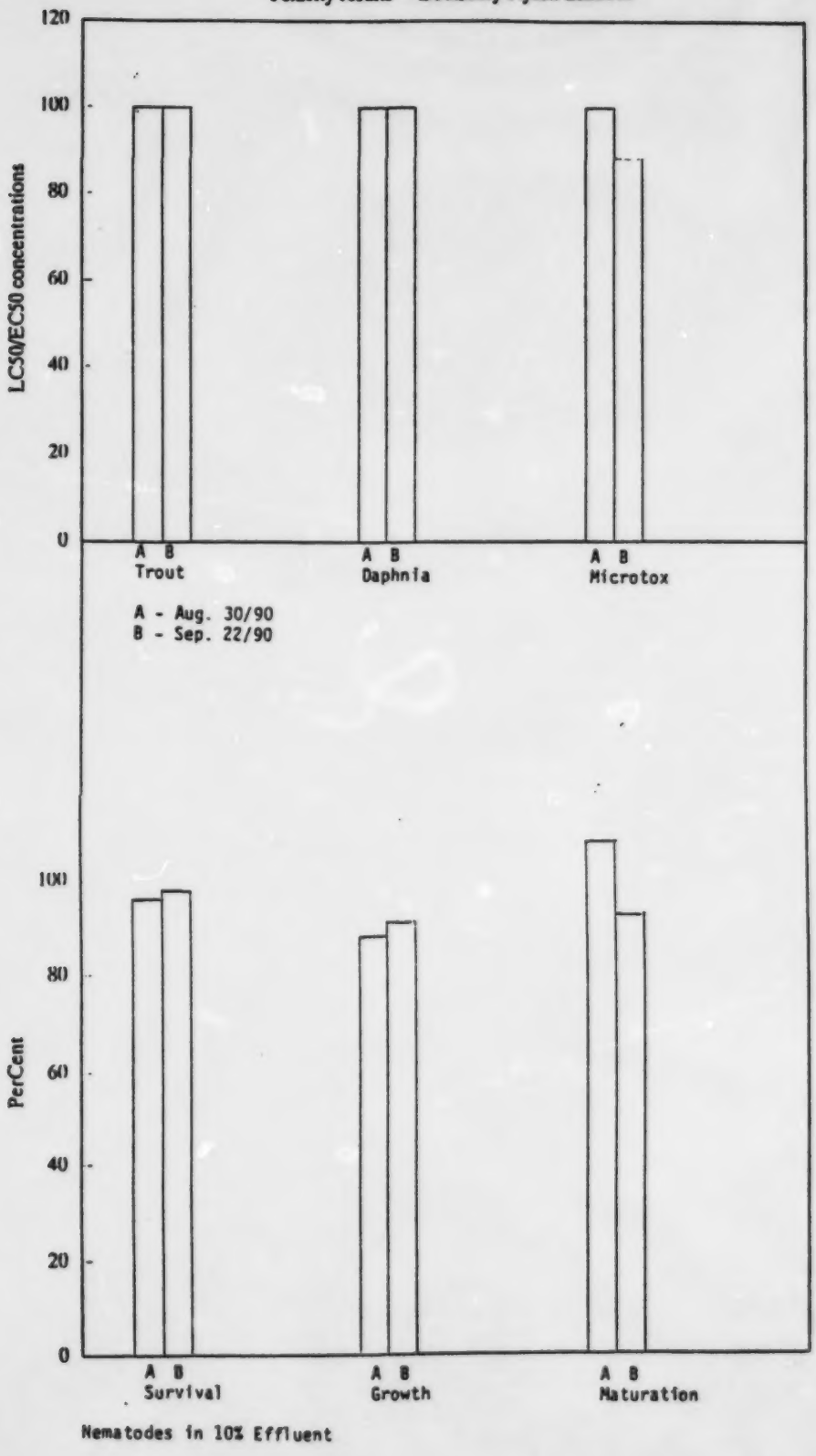
The storm sewer discharge is known to contain creosote, pentachlorophenol, polycyclic aromatic hydrocarbons, dioxins and furans which have as their origin two abandoned wood preserving sites in Prince Albert. Partitioning of these substances, their diverse solubilities, changes in groundwater levels and discharge rates may all influence the levels of these and associated but as yet unidentified toxic substance(s) in the effluent. Also, sampling of the outfall was difficult since the effluent passes through a hay dam which is replaced with new material periodically and this may have reduced the quantity of some toxic substances in the effluent. For these reasons it is expected that toxicity of this effluent may vary considerably from one sampling occasion to the next.

3.1.2.5 Boundary Flyash Effluent

Only two samples of this effluent were collected and analyzed (Table 2.1). The results are summarized in Figure 3.7.

Figure 3.7

Toxicity results – Boundary Flyash Effluent



No acute toxicity in either sample was observed in rainbow trout or Daphnia. In the Microtox test one sample showed no inhibition, the other only a modest inhibition of light emission (e.g. EC_{50} at 88%). No significant toxic effects were observed in nematode tests applied to either sample.

These results suggest little toxic influence of this effluent in the four bioassays employed. The effluent was not monitored further as it no longer is discharged into Boundary Reservoir - the drinking water supply source for the City of Estevan.

3.1.3 Municipal Sewage Effluents

Municipal sewage effluent from the Cities of Saskatoon, Prince Albert and Regina were examined for toxicity. One stream, Wascana Creek, was also sampled to determine if sewage effluent discharges from Regina caused measurable toxicity in this receiving stream.

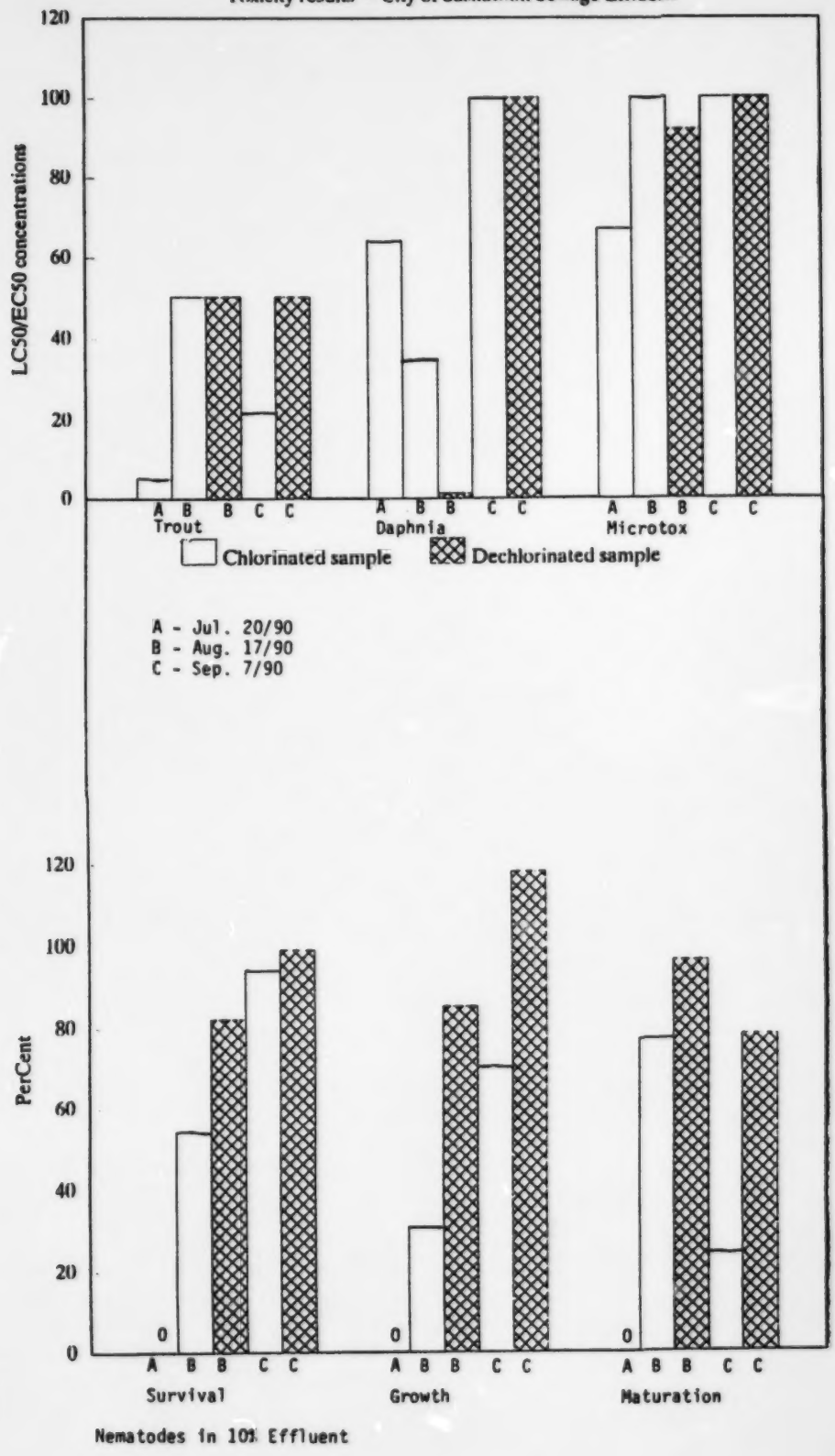
3.1.3.1 City of Saskatoon Effluent

A total of five final effluent samples were obtained from the Saskatoon municipal sewage treatment plant on three occasions in 1990 (Table 2.1). On two of these occasions (August 17 and September 7, 1990) duplicate grab samples were collected with one sample treated with sodium thiosulphate to neutralize residual chlorine while the other was left untreated. In each instance the chlorinated effluent sample and the dechlorinated sample were then shipped to the Winnipeg laboratory for toxicity analyses. The results are summarized in Figure 3.8.

The Saskatoon sewage effluent was acutely toxic to rainbow trout in all instances with the LC_{50}s occurring at concentrations of 5 to 50%. The July 20, 1990 sample was exceptionally toxic and at full strength (100% effluent) killed all trout within seconds of the fish being added to the test aquarium. On only one of two occasions (September 7, 1990) did sodium thiosulphate treatment of a sample appear

Figure 3.8

Toxicity results – City of Saskatoon Sewage Effluent



to reduce acute toxicity to trout yielding an LC_{50} at 50% compared to an LC_{50} of 22% for the untreated (chlorinated) sample.

Acute toxicity was also observed in the tests with Daphnia. Three of the five samples were toxic to Daphnia with LC_{50}s recorded at concentrations of effluent of 64, 35 and 1.2%. The most toxic sample (LC_{50} 1.2%) was the thiosulphate treated effluent collected on August 17, 1990. The duplicate effluent sample had an LC_{50} of 35%. The remaining two samples (September 7, 1990) were not acutely toxic to Daphnia.

In the Microtox test only the single sample of July 20, 1990 showed significant toxicity, yielding an EC_{50} value at a concentration of 67%. One other sample (August 17, 1990, dechlorinated) yielded an EC_{50} value of 92%. The two samples of September 7, 1990 displayed no toxicity in the Microtox test.

In the nematode test the July 20, 1990 sample was extremely toxic at the 10% concentration, killing all 100 test worms (e.g. 0 survivors). In the samples of August 17 and September 7, 1990 nematode survival, growth, maturation and fitness were significantly higher in the dechlorinated samples. For instance, on these two respective dates the survival of worms in 10% dilution of effluent was 54 and 94% in the chlorinated (untreated) sample, respectively. In the corresponding thiosulphate treated samples the rates of survival were 82 and 99%. There appeared to be significant benefit to worm survival, growth, and maturation imparted by sodium thiosulphate treatment of the chlorinated effluent samples to neutralize residual chlorine. The exact relationship of thiosulphate to these observations is uncertain, however, as similar trends were not apparent with other sewage samples treated in a similar manner (e.g. Figure 3.9) or where chlorinated and non-chlorinated effluents

were examined (Figure 3.10). There may be some unique characteristics related to toxicity of the Saskatoon sewage that are partially mitigated by treatment with sodium thiosulphate. Further testing of this should be done to confirm these observations.

In the 1% dilution of Saskatoon sewage no significant toxicity or inhibition of growth or maturation of the nematodes was observed in the four samples collected August 17 and September 7, 1990. This suggests that dilution to 1/100 its original concentration mitigated the toxic and inhibitory action of the sewage on the nematodes under the test conditions.

These results showed the Saskatoon sewage effluent to be acutely toxic in most tests and on all sampling occasions. On this basis the effluent did not meet the general water quality objective respecting effluent discharges (Saskatchewan Environment and Public Safety, 1988).

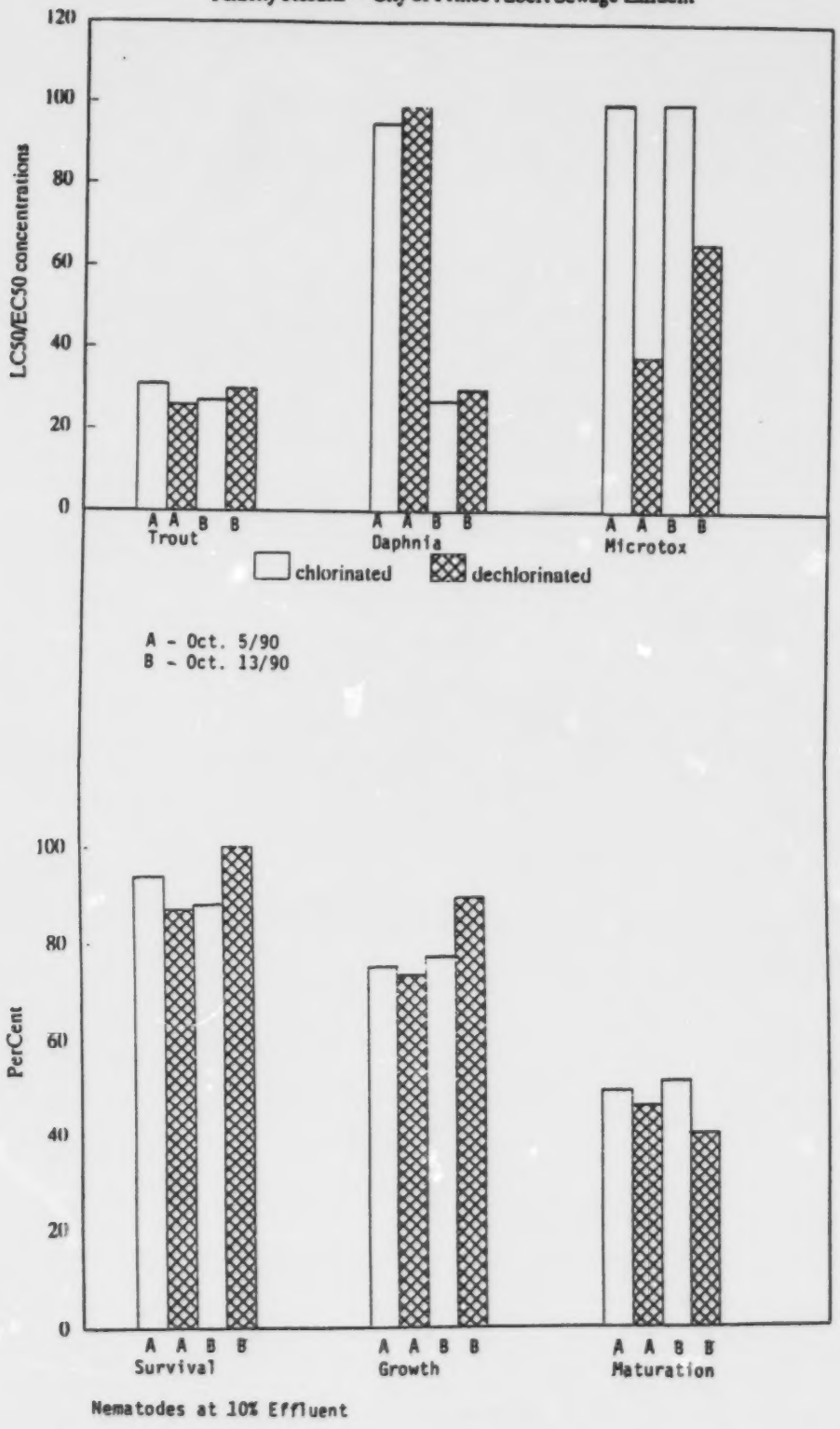
3.1.3.2 City of Prince Albert Effluent

A total of four samples were collected on two dates (October 5, 13, 1990) from the Prince Albert sewage treatment facility (Table 2.1). On each sampling occasion one sample of non-chlorinated effluent was obtained from the effluent leaving the plant clarifier. A second sample was then collected from the effluent leaving the chlorination facility to provide a chlorinated sample representative of chlorinated effluent discharged to the North Saskatchewan River during open water seasons. The results of analyses are summarized in Figure 3.9.

All four samples caused a similar level of acute toxicity to rainbow trout, with the LC_{50} values occurring at effluent concentrations in the range of 26 to 31%. There appeared to be no apparent difference in the acute toxicity of chlorinated and non-chlorinated samples of this effluent towards rainbow trout.

Figure 3.9

Toxicity Results – City of Prince Albert Sewage Effluent



Nematodes at 10% Effluent

The October 5, 1990 samples had little effect upon Daphnia (LC_{50} at 95 and 99%) while the October 13, 1990 samples were very toxic to Daphnia with LC_{50} values obtained at effluent concentrations of 27 and 30% in the non-chlorinated and chlorinated samples, respectively.

The Microtox test results are of interest for they indicate the non-chlorinated samples caused no toxicity in this test (e.g. EC_{50} values 100%). The chlorinated effluent samples of October 5 and 13, 1990, however, yielded EC_{50} values at effluent concentrations of 38 and 66% respectively.

Unlike the observations made with respect to chlorinated and dechlorinated (thiosulphate-treated) Saskatoon sewage (Section 3.1.3.1) there were no apparent trends respecting differences between the survival, growth, or maturation of nematodes in the Prince Albert chlorinated and non-chlorinated effluent samples. Three of the effluent samples caused

slight acute toxicity in nematodes with survival levels ranging from 87 to 94% in the 10% dilutions of effluent. One sample (chlorinated effluent of October 13, 1990) had no acute toxicity (e.g. 100% survival). Nematode growth values ranged from 73 to 89% while maturation was inhibited to a greater extent in all samples with values ranging from 40 to 51%.

In the 1% dilutions of effluent there was nearly no loss of nematodes (nearly all survived) and slight inhibition of growth and maturation. Chlorinated samples from both dates showed significantly greater inhibition of nematode maturation (e.g. 59 and 65%) compared to the non-chlorinated effluent samples.

The Prince Albert sewage effluent sampled in October, 1990 was shown to be acutely toxic to rainbow trout and Daphnia and to cause inhibition of growth and maturation in nematodes. The effluent did not meet the water quality objective respecting

effluent acute toxicity (Saskatchewan Environment and Public Safety, 1988).

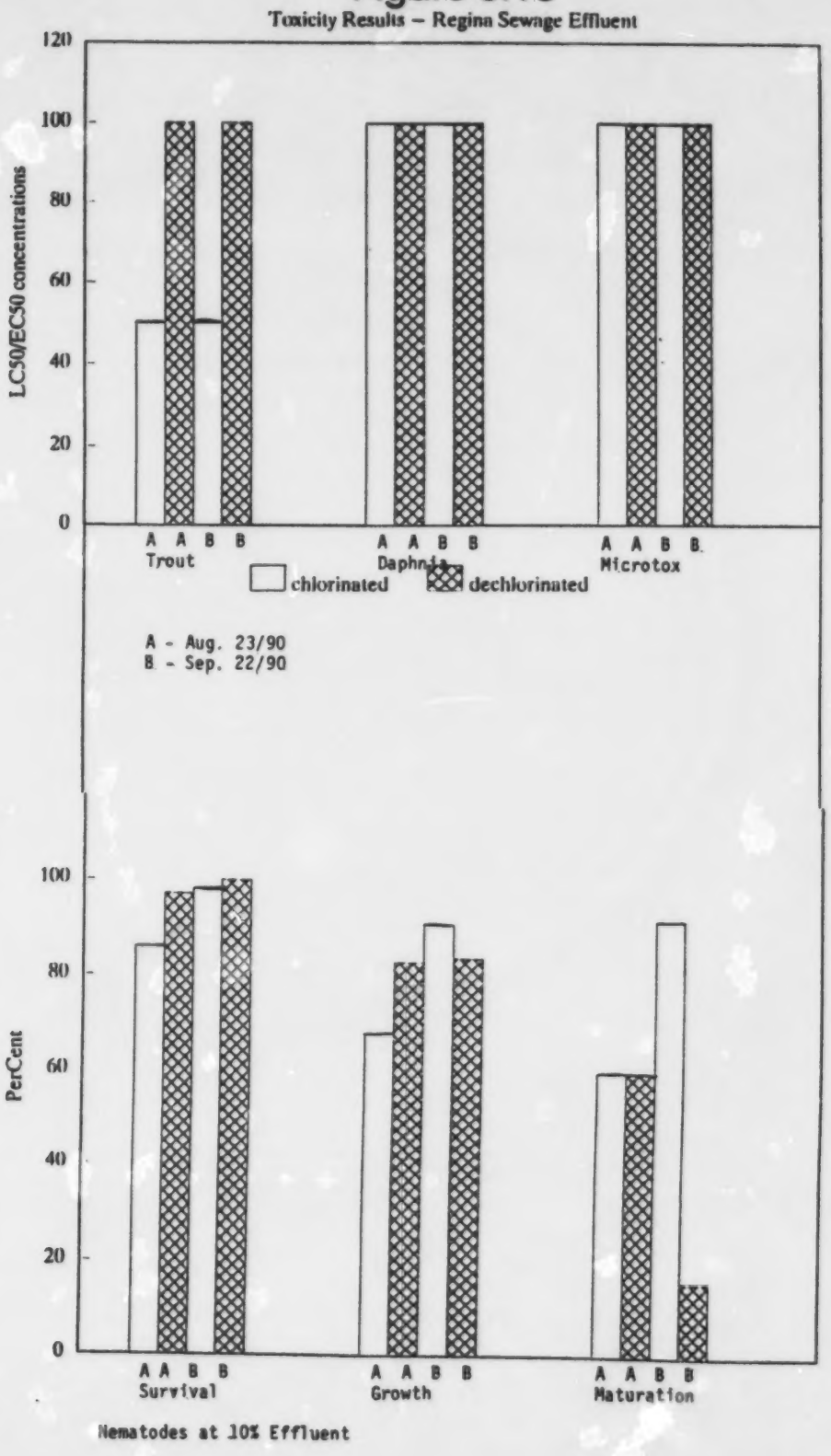
3.1.3.3 City of Regina Effluent

The tertiary treated, disinfected effluent of the City of Regina was sampled on two occasions (Table 2.1) in the fall of 1990. On each occasion one of two duplicate effluent samples was treated with sodium thiosulphate to neutralize residual chlorine. The results of analyses are shown in Figure 3.10.

The chlorinated effluent samples of August 23 and September 22, 1990 caused acute toxicity in rainbow trout. In both cases the LC_{50} value occurred at the 50% concentration of effluent. Treatment of these chlorinated effluent samples with sodium thiosulphate mitigated the acute toxicity to rainbow trout completely.

No sample caused acute toxicity to Daphnia or inhibition of light emission in the Microtox tests. In other words both of these bioassays indicated the effluent samples to be non-acutely toxic.

Figure 3.10


Toxicity Results – Regina Sewage Effluent
LC50/EC50 concentrations
120
100
80
60
40
20
0
A A B B
Trout
A A B B
Daphnia
A A B B
Microtox
chlorinated
dechlorinated
A - Aug. 23/90
B - Sep. 22/90
PerCent
100
80
60
40
20
0
A A B B
Survival
A A B B
Growth
A A B B
Maturation
Nematodes at 10% Effluent

Only one sample, chlorinated effluent of August 23, 1990, caused slight (86% survival) acute toxicity in the nematode test. Also in this sample and its dechlorinated (thiosulphate treated) duplicate sample significant inhibition of growth and maturation was noted. In the September 22, 1990 dechlorinated sample at a 10% dilution only 16% nematodes reached maturity compared to 92% in the untreated chlorinated sample.

Nematodes tested in the 1% dilutions of effluent showed 99% survival and only very slight inhibition of growth and maturation.

These bioassay results indicate that the Regina sewage effluent was much less acutely toxic than were either of the sewage effluents discharged by Saskatoon or Prince Albert. They also indicate that sodium thiosulphate eliminated acute toxicity to rainbow trout in this effluent. Also, it indicates the value of

using several bioassays to assess toxicity since two of the tests (Daphnia and Microtox) did not indicate any acute toxicity.

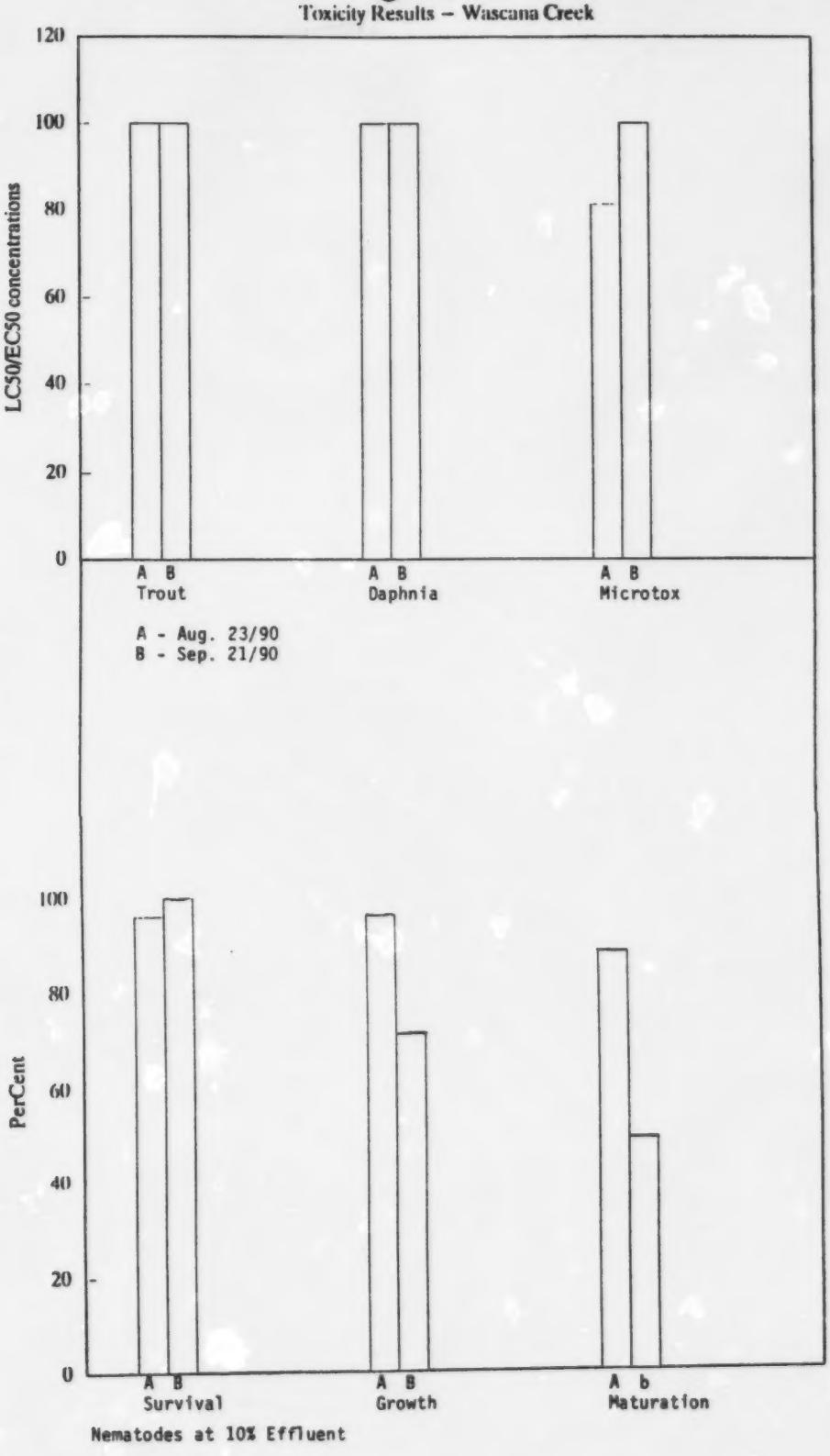
3.1.3.4 Wascana Creek

Wascana Creek receives treated sewage effluent from the City of Regina sewage treatment facility. Sewage effluent in this creek may contribute significantly to the flow in this waterbody, at times making up one-half or more of the water in the creek. The results of toxicity testing of creek water collected on August 23 and September 21, 1990 are summarized in Figure 3.11.

Neither creek water sample showed acute toxicity for rainbow trout or Daphnia (e.g. LC_{50} values all >100%). The August 23, 1990 sample caused moderate light inhibition in the Microtox test (EC_{50} at 81%). The September 21, 1990 sample caused no inhibition of light emission in this test and would be considered non-toxic on the basis of this test result.

Figure 3.11

Toxicity Results – Wascana Creek



Nearly all nematodes survived exposure to 10% dilutions of both creek samples. Survival values of 96 and 100% were recorded indicating no acute toxicity. The September 21, 1990 sample caused some inhibition of growth (71% grew) and maturation (49% matured) which might be considered a possible indicator of chronic toxicity occurring in the creek.

These results indicate no significant acute toxicity was associated with Wascana Creek water samples collected in August and September, 1990.

3.2 Analytical Test Assessment

In this study a total of 37 surface water and effluent samples were analyzed for acute toxicity/inhibitory activity using four bioassays as a battery. The results of these analyses were reported above in Section 3.1 and discussed in terms of the acute toxicity or inhibitory property of the various effluents or water samples. In this section the four tests are evaluated on the basis of their sensitivity to the waters and effluents.

3.1.1 Reactions to Control Samples

In this study the assumption was made that the control monitoring sites, being free of known significant effluent discharges or diffuse pollutional sources, would not produce acute toxic responses in the bioassays selected for assessment. For instance, LC_{50} and EC_{50} values would generally be expected to be at concentrations exceeding 100% of the sample concentration. Six samples from two control sites were evaluated and the results summarized in Table 3.1. In this and the following section concerning effluents a positive reaction in each bioassay was considered to be an LC_{50} or EC_{50} value that was obtained at a sample concentration of less than or equal to 95%. Similarly, in the nematode test an acute response was considered to have occurred where less than or equal to 95% of test organisms survived exposure to the 10% sample concentration. A positive chronic response was considered to have occurred when 95% or less of the test worms showed growth or maturation.

As shown in Table 3.1 none of the six samples displayed acute toxicity for rainbow trout or for *Daphnia*. One sample from the South Saskatchewan

Table 3.1

SUMMARY OF TOXIC REACTIONS DETECTED IN CONTROL SAMPLES

Control Sample Source	Number Samples	LC_{50}/EC_{50} at ≤95% Conc.			Nematodes * in 10% sample		
		Rainbow Trout	Daphnia	Microtox	Survival	Growth	Maturation
Red Deer River	1	0	0	0	1	1	1
South Saskatchewan River	5	0	0	1	0	4	4

* Number samples where ≤95% of nematodes survived, grew, matured.

River caused some inhibition of light emission in the Microtox test (EC_{50} at 75%) and the Red Deer River sample caused reduction in survival of nematodes (82% survived) and also impaired growth (23% grew) and maturation (60% matured). No significant reduction in nematode survival occurred with the 10% South Saskatchewan River samples but four of the five samples were inhibitory to nematode growth and maturation. One might view this a false positive reaction respecting growth/maturation that may be due to unidentified non-toxicity related characteristics/conditions of the test solutions (e.g. ionic changes, starvation, infection?). Conversely, one might consider the results to be due to actual toxicity. However, this is difficult to explain in view of the generally high quality of the water and protection from pollution at these monitoring sites.

These unexplained results obtained with the Microtox and nematode tests in these control samples need to be considered when reviewing results of effluents analyzed by the same procedures, particularly when only slight acute toxicity (e.g. LC_{50} or EC_{50} values at very high effluent concentrations) is recorded.

On the basis of the few samples (6) analyzed as control water sources, possibly false positive acute toxicity results may have been observed for the Microtox and nematode bioassays only. False positive results at this frequency of occurrence in two tests of a battery of four test protocols would not, however, not represent a major obstacle to the assessment of the acute toxicological characteristics of environmental water samples. It represents less of a concern than if false negative results were found to occur. False negative results were not recorded for any of these bioassays in quality assurance procedures conducted at the analytical laboratory.

In future studies some control samples which are presumed to be non-toxic should be spiked with a quantised amount of toxic substance(s) and submitted for analyses by Microtox and nematode bioassay. This would help reduce the subjectivity regarding perceived false positive results with these two bioassays.

3.2.2 Reactions to Effluent Samples

A total of 29 effluent and two receiving water samples were analyzed by the battery of four bioassays. The total number of sample results of

these 31 samples were evaluated with respect to the number of significant detections of toxicity inhibition which were recorded for each bioassay procedure. As was done with respect to the control site samples, a positive acute toxicity result was considered to have occurred when LC_{50} or EC_{50} values were obtained at effluent concentrations of 95% or less. In the nematode test, a survival rate of 95% or less was considered significant and scored as an acute toxic result. Growth or maturation of 95% or less of the nematode test population was considered indicative of chronic toxicity or inhibition due to sample toxicity. The results are summarized in Table 3.2.

Of the four tests conducted, acute toxicity was observed more frequently (18 of 31 samples or 58%) in rainbow trout than in the other three bioassays. Sewage effluents were generally more acutely toxic to trout with 11 of 13 (85%) samples being acutely toxic compared to 7 of 16 (44%) of industrial effluent samples. This may relate in part to the irregular or high variability believed to characterize the quality (chemical/physical) of several of the industrial effluents where little

Table 3.2

SUMMARY OF TOXIC REACTIONS DETECTED IN EFFLUENT SAMPLES

		Reactions in Various Tests									
		LC_{50} at ≤95%		EC_{50} at							
Effluent Source	Number Samples Tested	Rainbow Trout	Daphnia	≤95% Microtox	Nematodes ** in 10% Effluent Survival	Growth	Maturation	Number Samples	Nematodes ** in 10% Effluent Survival	Growth	Maturation
Akzo Chemicals	(3)	2	3	3	2	2	3	(2)	0	1	1
Saskatoon Chemicals	(3)	2	1	2	1	3	2	(2)	1	1	2
Weyco	(4)	1	0	1	2	2	3	(3)	1	0	0
8th Avenue East Storm Sewer	(4)	2	1	2	1	3	4	(3)	0	1	0
Boundary Flyash	(2)	0	0	1	0	2	1	(1)	0	1	0
Saskatoon Sewage	(5)	5	3	2	4	4	4	(4)	1	1	2
Prince Albert Sewage	(4)	4	3	2*	3	4	4	(4)	0	3	4
Regina	(4)	2	0	1	1	4	4	(4)	0	2	2
Wascana Creek	(2)	0	0	1	0	1	2	(0)	--	--	--
Totals	(31)	18	11	15	14	25	27	(23)	3	10	11

* both chlorinated

** samples where ≤95% of nematodes survive, grew, matured.

treatment or retention of wastewater from a variety of effluent streams occurs (e.g. Akzo and Saskatoon Chemicals).

Acute toxicity was observed in 11 (35%) of samples tested using Daphnia, 15 (48%) using Microtox and in 14 (45%) samples where nematode survival was determined. Inhibition of growth and/or maturation (chronic toxicity) was prevalent in most effluent samples occurring at the frequencies of 81% (25 of 31 samples) and 87% (27 of 31 samples), respectively. Maturation appeared, in general, to be the most sensitive measure of toxic inhibition in both frequencies of occurrence and magnitude of the inhibition (e.g. generally higher rates of inhibition of maturation). Since nematodes were tested at 10% of the original sample concentration these acute and chronic toxicity response likely grossly underestimate the toxicity that would have occurred with the raw sample material.

In the 1% effluent dilutions approximately 13% of samples caused acute toxicity to nematodes, whereas 40 to 48% of these same samples resulted in chronic toxicity (inhibition of growth/maturation). These

results suggest the nematodes remained sensitive to these effluents despite a 100:1 dilution of test sample.

At present, these results provide an initial indication that no single test can reliably determine the acute toxicity of any of the effluent types examined in this study. While there was no single effluent, except for the Boundary Flyash (Figure 3.7), which was not acutely toxic on at least one occasion, some of the results in which acute toxicity was not observed may have related to the high variability in chemical or physical quality of some of the effluents.

The numbers of samples taken from various effluent sources did provide evidence that all municipal sewage and most industrial effluents were acutely toxic and on occasion very acutely toxic, in one or more of the four tests applied to them. Because of effluent quality variability, lack of wastewater treatment and/or retention in some instances and difficulties in sampling some sites in a uniform manner (e.g. 8th Avenue East storm sewer) it is difficult to make comparisons between the results

recorded for the four bioassays. The individual sample results for each bioassay are summarized in Appendix 3 to tally those results showing significant acute toxicity or inhibition and those which did not.

The overview of results indicates the following performance of the battery of tests:

1. Six samples yielded positive results in all four tests;
2. Five other samples yielded positive results in three tests;
3. Seven other samples yielded positive results in two tests;
4. Four samples yielded positive results in only one test;
5. Nine samples yielded positive results in none of the tests;
6. In the 18 samples where acute toxicity occurred in rainbow trout 10 caused acute toxicity for Daphnia, 9 caused acute toxicity in Microtox and 13 caused acute toxicity in nematodes (95% or less survived in the 10% concentration);

7. In four samples which were non-acutely toxic to rainbow trout acute toxicity was observed in all four samples in the Microtox test, and in one sample each for the Daphnia and nematode survival test;
8. Of the 31 effluent samples 26 (84%) elicited chronic toxicity in nematodes' (at the 10% concentration) growth and in 28 of 31 (90%) significant impairment of maturation was recorded.

These results suggest that the battery of tests approach to at least the initial toxicological assessment of effluents is necessary to increase the likelihood of obtaining a measure of acute or chronic toxicity with one or more than one of the test protocols utilized. The nematode test, since usually carried out at a 10% concentration of effluent likely would have provided a far higher incidence of acute toxicity if tests had been conducted at higher effluent concentrations (e.g. 50%, 90%, etc.). However, at higher effluent concentrations the expected increased lethality would result in loss of information about growth and maturation for that higher test concentration. It may therefore be beneficial to conduct some

analyses in future surveys at several concentrations to obtain both acute (lethality) and chronic (growth/maturation) toxicity information.

The Daphnia magna acute lethality test and the rainbow trout test have been proposed by the federal environmental agency in Canada as regulatory tools to assess acute toxicity in pulp mill effluents. In the four samples obtained from the mill at Prince Albert only two elicited any acute toxic response in the battery of tests with rainbow trout (1), Microtox (1) and nematodes (2). D. magna showed no acute toxic response to these samples. As more toxicity data is obtained it may be possible to confidently delete or add specific test protocols to the battery of tests applied to individual effluents to provide greater assurance that false negative results due to insensitive tests are not obtained.

ACKNOWLEDGEMENTS

The study was co-ordinated and the report prepared by K. P. Lauten. Grateful appreciation is expressed to Dr. M. Samoiloff for conducting the laboratory analyses and for his generous assistance in introducing the author to the "art" and science of toxicology. M. D. Hilderman and R. H. Zitta provided logistical support for the study and carried out much of the monitoring and provided assistance in the preparation of figures as well as offering review comments respecting the manuscript. R. G. Ruggles and D. D. Nargang provided valuable review comments and suggestions for preparing the final report.

REFERENCES

Lauten, K. P. 1990 (unpublished) Aquatic Toxicity Tests: Application of Selected Methods for Water Quality Monitoring of Effluents in Saskatchewan. April 1990, 28 pp.

Saskatchewan Environment 1983. Water Quality Review and Assessment of Boundary Reservoir. WPC 32, May 1983, 122 pp.

Saskatchewan Environment 1984a. Wascana Creek/Qu'Appelle River Bacteriological Quality - Summer 1982. WPC 39, July 1984, 86 pp.

Saskatchewan Environment 1984b. Water Quality Study - North Saskatchewan River - Prince Albert Area (Autumn 1983). WPC 40, Sept. 1984. 123 pp.

Saskatchewan Environment and Public Safety 1988. Surface Water Quality Objectives. WQ 110, November 1988, 33 pp.

Saskatchewan Environment and Public Safety 1991. Akzo Chemicals and Saskatoon Chemicals Mixing Zone Study. South Saskatchewan River at Saskatoon. July and September 1988. In press.

Tones, P. I. 1981. Regina Tertiary Treatment Plant - Effects on Water Quality in Wascana Creek. WPC 28, 253 pp.

LIST OF APPENDICES

Appendix 1	Toxicity Test Protocols
Appendix 2	Results of Nematode Tests at One Per Cent Effluent Concentration
Appendix 3	Summary of Toxicity Test Results for Effluents

APPENDIX 1

TOXICITY TEST PROTOCOLS

APPENDIX I

PROPOSED CHECKLIST FOR RAINBOW TROUT ASSAY

TABLE 2

CHECK-LIST OF RECOMMENDED TEST CONDITIONS AND PROCEDURES

UNIVERSAL

Test type:	Static, 96-h duration[5].
Dilution and control water:	Ground, surface, or dechlorinated municipal water. *Upstream receiving water to assess toxic impact at a specific location[6]. Reconstituted water if requiring a high degree of standardization. Dissolved oxygen (DO) content 90-100% saturation at time of use.
Fish:	Swim-up fry or fingerlings, 0.3 - 5 g. Normally a minimum of 10/test solution. Fish-loading density ≤0.5 g•4d/L.
Solution depth:	≥15 cm.
Temperature:	15 ± 1 C.
Oxygen/aeration:	Pre-aerate test solutions for ≤120 min at ≤7.5 ml/min•L if DO of one or more solutions <7 mg/L. Aerate solutions at this rate during test.
pH:	No adjustment if pH of test solution within the range 5.5 to 9.5[7]. A second (pH-adjusted) test may be required or appropriate if sample/solution pH beyond this range.
Lighting:	Full-spectrum fluorescent, 20 - 30 lux at surface, normally 16-h light:8-h dark, preferably gradual transition.
Feeding:	Do not feed for at least 12 h before start of test, nor during test.
Observations:	Fish death, appearance and behaviour. At least at 24, 48, 72 and 96 h.
Measurements:	Temperature, pH, and DO; at least at start and end (preferably daily).
End-points:	As specified and/or dependent upon test objectives and test material. May be 96-h LC50 (requiring 95% confidence limits) or single-concentration test (% mortality at 96 h or earlier; LT50).

---

[5] Special situations (e.g. volatile or unstable chemicals in solution) may require the use of flow-through or static replacement tests, or a modified test duration.

[6] If receiving water is used as the dilution and control water, an additional control is required using the uncontaminated laboratory water supply to which fish were previously acclimated.

[7] If pH is outside this range, results may reflect toxicity due to biologically-adverse pH.

Reference toxicant:	Phenol and/or zinc (as zinc sulfate). Conduct static 96-h LC50.
Test validity:	Invalid if control mortality >10%.

CHEMICALS

Solvents:	To be used only in special circumstances.
Concentration:	Recommended to be measured at beginning and end of exposure, in high, medium and low strengths and in the control(s). If concentrations decline ≥20%, re-evaluate by flow-through or static replacement test.
Dilution and control water:	As specified and/or dependent upon intent. Reconstituted if require high degree of standardization; receiving water if concerned with local toxic impact; otherwise, laboratory water.

EFFLUENTS AND LEACHATES

Transport and storage:	Transport at ambient temperature (>1 C, <30C) or at 1-8 C if transit time >2 d. Test may be invalid if sample frozen or partially frozen during transit. Store at 4 ± 2 C. Test within 3 days of sampling.
Dilution and control water:	As specified and/or dependent upon intent. Laboratory water or "upstream" receiving water for monitoring and compliance.
High solids or floatables:	May choose to recirculate test solutions.

ELUTRIATES

Transport and storage:	Extract within 7 d of sample receipt. Test within 10 d of sample receipt.
Dilution and control water:	As specified and/or dependent upon intent. Reconstituted water if a high degree of standardization is required.

RECEIVING WATER

Transport and storage:	As for effluents and leachates.
Dilution and control water:	As specified and/or dependent upon intent. If studying local impact use "upstream" receiving water as control and dilution water.

APPENDIX II

PROPOSED CHECKLIST FOR *DAPHNIA* ASSAY

TABLE 3

CHECK-LIST OF RECOMMENDED TEST CONDITIONS AND PROCEDURES FOR ACUTE LETHALITY TESTS USING *Daphnia* sp.

UNIVERSAL	
Test type:	Static, 48-h duration[13].
Dilution and control water:	Reconstituted water, particularly if a high degree of standardization is desired. Uncontaminated ground, surface, or dechlorinated municipal water. "Upstream" receiving water to assess toxic impact at a specific location[14]. Dissolved oxygen (DO) content 90-100% saturation at time of use.
Organisms:	Neonates of *Daphnia magna* or *D. pulex*. ≥10 daphnids per concentration. *D. pulex* required if testwater hardness <50 mg/L. Loading density, ≤one daphnid/15 ml.
Temperature:	20 ±2 C.
Oxygen/aeration:	No aeration. Special interpretation if DO <60%.
pH:	No adjustment if pH of test solution is within the range 5.5 - 9.0[15]. A second (pH-adjusted) test may be required or appropriate if pH of sample/solution is beyond this range.
Lighting:	Preferably "cool white" fluorescent, ≤800 lux at surface, normally 16-h light:8-h dark.
Feeding:	Do not feed during test.
Observations:	Mortalities and atypical behaviour (e.g. immobility, lethargy, circling, floating) observed at beginning and end of test, as a minimum.
Measurements:	Solution temperature, pH and DO, at least at beginning and end. Hardness to be assessed, conductance desirable.

---

[13] Special situations (e.g. unstable chemicals) may require continuous-flow or static replacement tests, or a change in duration.

[14] If receiving water is used for dilution, an extra control must use the laboratory water in which daphnids were cultured.

[15] If pH is outside this range, results may reflect toxicity due to biologically-adverse pH.

End-points:	As specified and/or dependent upon test objectives and material. May be 48-h LC50 (requiring 95% confidence limits) or single-concentration test (% mortality at 48 h or earlier; LT50).
Reference toxicant:	One or more of zinc sulphate, sodium chloride, or potassium dichromate. Static 48-h LC50, determined within 10d of test.
Test validity:	Invalid if control mortality >10%.

CHEMICALS

Solvents:	To be used only in special circumstances.
Concentration:	Desirable to measure at beginning and end of exposure, in high, medium and low strengths and in control(s). If concentrations decline ≥20%, re-evaluate by flow-through or static replacement test.
Dilution and control water:	Dependent upon intent. Reconstituted if require high degree of standardization; receiving water if concerned with local toxic impact; otherwise, uncontaminated laboratory water.

EFFLUENTS AND LEACHATE

Transport and storage:	Transport at 1-8 C. Test may be invalid if sample frozen or partially frozen during transit. Store at 4 ±2 C. Test within 3d of sampling if possible, must be tested within 5 days of sampling.
Dilution and control water:	Dependent upon intent. Laboratory water, reconstituted water, or "upstream" receiving water for monitoring and compliance.

ELUTRIATES

Transport and storage:	Extract within 7d and test within 10d of sample receipt.
Dilution and control water:	Reconstituted water unless specified otherwise.

RECEIVING WATER

Transport, storage:	As for effluents and leachates.
Dilution and control water:	Dependent upon intent. If studying local impact use "upstream" receiving water as control and dilution water.

APPENDIX III

DETAILS OF THE *PANAGRELLUS REDIVIVUS* TEST

(Biological Assessment. 1990. In Press)

The Nematode Toxicity Assay Using *Panagrellus redivivus*

MARTIN SAMOILOFF, *Bioquest International Incorporated, 7 Loyola Bay, Winnipeg, Manitoba R3T 3J7*

1.0 OBJECTIVE

This test is intended to evaluate the impact of contaminants in aqueous samples on several biological endpoints in a quantitative manner.

2.0 SCOPE

The nematode test has been utilized on the following types of samples:

(i) Water samples from lakes, rivers, streams, ground water, holding ponds, tailing ponds, and treatment ponds.

(ii) Aqueous extracts of soils, sediments, sludges, consumer products, and chemical absorbents.

(iii) Methanolic extracts of soils, sediments, sludges, consumer products, and chemical absorbents.

(iv) Organic extracts from soils, sediments and sludges, exchanged into dimethylsulphoxide (DMSO).

3.0 PRINCIPLE

3.1 The nematode *Panagrellus redivivus* contains approximately 530 somatic cells, organized into tissues and organs. The animal undergoes live-birth, with the newborn animals designated as second-stage juveniles (J2s). Over a 96 hour period, the newborn J2s grow through two additional juvenile stages (J3 and J4) to the adult stage. Each stage of *P. redivivus* falls within a characteristic size range. Under adverse conditions, the animals will arrest their growth,

3.2 Monitoring a population of 100 J2 animals over the 96 hour growth period provides a quantitative method of measuring both lethal and sublethal effects of a tested sample. Lethal effects are determined by the reduction in the total number of animals in the population. The number of animals remaining at the J2 or J3 stages provides a measure of sublethal effects.

3.3 Growth from J2 to J3, or from J3 to J4 requires very little gene activity, while growth from J4 to adult requires extensive gene activity. Many known mutagens will selectively inhibit the J4 to adult moult, and specific inhibition of growth of J4s to adults can be used as an indicator of potential mutagenicity in the test sample. The test is not a direct test for mutagenicity, but can be used as a "flag" to indicate a requirement for further mutagenicity testing.

4.0 REAGENTS, MATERIALS AND APPARATUS

4.1 Reagents

Use reagent grade chemicals and deionized distilled water (or equivalent).

4.1.1 M9 Buffer

Sodium phosphate, (dibasic) $Na_2HPO_4 \cdot 7H_2O$	6.00 g
Potassium phosphate, (monobasic) KH_2PO_4	3.00 g
Sodium chloride, NaCl	5.00 g
Magnesium sulphate, $MgSO_4$	0.25 g
Distilled water	1.00 L

Autoclave at 121° C for 15 minutes. M9 Buffer is a non-nutritive liquid used for washing animals, limiting growth, and providing a standard environment.

4.1.2 Cholesterol Solution

cholesterol	500 mg
ethanol	100 mL

The solution is gently heated until the cholesterol is dissolved. This solution provides required steroid to all growth media.

4.1.3 *Yeast Suspension*

Dried Baker's yeast	50 mg
Distilled water	100 mL

Approximately 4 mL of suspension is distributed to a series of 7 mL vials which are autoclaved for 15 minutes at 121°C. This suspension is used as a food source for growing cultures.

4.1.4 *M9-Y Growth Medium*

M9 Buffer	99.0 mL
Yeast Suspension	1.0 mL
Cholesterol Solution	0.1 mL

The mixture of M9 buffer and yeast suspension is autoclaved for 15 minutes at 121°C. After autoclaving, the cholesterol solution is added to the hot mixture. The M9-Y medium is a limited nutrient medium used for transfer of stocks to fresh plates, for limited growth of small nematode populations, and as the basic medium for bioassays.

4.1.5 *Water-Agar Plates*

Agar	17 g
Distilled water	1 L
Cholesterol solution	1 mL

The mixture of agar and water is autoclaved for 15 minutes at 121°C, and 1 ml of cholesterol solution is added to the hot agar. Approximately 20 mL of agar is poured into each of a series of 100 mm diameter plastic Petri plates. After cooling and solidifying, the plates are stored in a refrigerator.

These plates are used to maintain small populations of stock cultures and to collect broods of J2 animals.

4.1.6 *Mass Culture Medium*

Buckwheat or Rye flour	100 mL
Distilled water	85 mL

The flour and water are mixed in a 1 quart Mason jar, and the jar is covered with a 100 mm glass Petri dish top. The mixture is autoclaved at 121°C for 15 minutes.

4.2 Materials

100 mm diameter Petri plates
AutoAnalysis sample cups (2.5 mL, Flat bottom) and caps
Holders for AutoAnalysis sample cups (ideally holding 10 rows of 10 cups each)
Glass Pasteur pipettes
rubber bulbs for pipettes.
Glass microscope slides, "subbed" by dipping in a hot solution of 1% gelatin containing 0.1% chromium potassium sulphate, and permitting the slides to dry before use.
Cotton-blue lactophenol stain

4.3 Apparatus

dissecting microscope (working power of approximately 30X)
microscope with eyepiece measuring reticule (working magnification of approximately 100 X)
Microscope slide warmer or 60°C hot plate

5.0 CULTURE MAINTENANCE

Stocks of *Panagrellus redivivus* strain bq-1 can be obtained from Bioquest International.

5.1 Stock Culture Maintenance

5.1.1 Mass cultures of Panagrellus are maintained in quart mason jars containing mass culture medium (4.1.6). A small population (several hundred individuals) nematodes in M9 buffer are added to the flour water mixture. After several weeks, swarms of nematodes can be observed on the walls of the jar. Populations in excess of 50,000,000 individuals can be maintained for several weeks in these cultures. Routinely, new cultures are prepared twice monthly.

5.1.2 Lower population density stock cultures of *P. redivivus* are maintained on water-agar plates. These cultures are fed every 4-5 days with three drops of yeast suspension per plate. Subcultures are obtained by flooding older plates with approximately 10 mL of M9-Y solution, and transferring 3-4 mL of the fluid containing the nematodes to fresh water-agar plates.

5.2 Cultures for Toxicity Tests

5.2.1 Toxicity tests are performed using J2 animals. Before each test is performed, an adequate supply of J2 animals is a similar physiological state must be obtained.

5.2.2 The day before a bioassay is to be performed, a brood plate is prepared. Gravid females are transferred from a stock culture to a fresh water-agar plate. Gravid females are large, fat animals with a very granular midbody region, due to eggs, embryos, and J1 animals within the female.

5.2.3 There are two ways that the transfer of gravid females can be performed.

(i) The stock plate can be flooded with M9 buffer, and individual females picked up and transferred by micropipette, or
(ii) the females can be picked up from the dry agar surface using a sharpened applicator stick.

In both cases the fresh plate containing only gravid females should be flooded to a depth of 2-4 millimetres with M9 buffer. These females will produce J2 animals. In the absence of food these J2 animals will not grow. Each female will produce 10-20 J2 offspring in a 12 hour period. These offspring, arrested at the J2 stage are used in the *P. redivivus* bioassay.

6.0 TESTING PROCEDURE

6.1 For bioassays, 10 mL aliquots of tested material diluted in M9-Y medium, are prepared. For standard bioassays using either aqueous samples, methanolic extracts of samples, or sample extracts exchanged in dimethylsulphoxide (DMSO), the following dilutions are routinely used:

A. Aqueous samples (10%):
1.0 mL sample + 9.0 mL M9-Y medium

B. Methanol extracts (3%):
0.3 mL sample + 9.7 mL M9-Y medium

C. DMSO samples (1%):
0.1 mL sample + 9.9 mL M9-Y medium

6.2 Control medium is prepared by diluting the appropriate carrier (water, methanol, DMSO) in M9-Y medium to the same level as the tested material. For each test set, a food control, containing only m9-Y medium should also be set up.

6.3 The diluted samples are placed in 2.5 mL flat bottom AutoAnalysis cups. Each standard test or control uses 0.5 mL of tested material in each of 10 cups for each test sample or control.

6.4 Larvae from the brood plate are transferred to the test and control cups. This transfer requires manipulation of the animals using a micropipette. The micropipette is prepared by heating a glass Pasteur pipette in a flame until the glass becomes soft, then removing the pipette from the flame and pulling the two ends of the pipette in order to draw out a fine tip.

6.5 Using the micropipette, 10 J2 animals are picked up from the plate containing gravid females and larvae, and placed in each control or test AutoAnalyzer cup. Each test and control group will consist of 10 AutoAnalysis cups, each containing 10 J2 animals. The cups are capped after the animals are placed in the diluted test or control medium.

6.6 The animals are permitted to grow over a 96 hour period. Growth temperatures between 19° C and 25°C are acceptable.

6.7 After the 96 hour growth period, the cups are opened, and the number of survivors in each plate is recorded. The survivors from each group are removed from the cups, and placed on a "subbed" slide on a 60 degree Celsius hot plate. All of the survivors from the 10 cups of one test are placed in a drop no more than 1 cm in diameter on the subbed slide. The drop is allowed to evaporate to dryness, and the heat killed, stretched out nematodes are stained with a drop of cotton blue- lactophenol. The stained animals are covered with a 22 mm square glass coverslip.

6.8 The length of the stained animals is used to establish the stage distribution. The length can be determined by measuring through a microscope, by using a microscope mounted video camera, or by preparing a hardcopy of the slide using a microfiche reader/printer and determining length with either a map reader or a graphics tablet.

6.9 The number of animals in each stage for each test and control set is recorded. The size range for each stage is:

(i)	J2	250 - 350 micrometers
(ii)	J3	350 - 550 micrometers
(iii)	J4	550 - 750 micrometers
(iv)	adult	750 - 2000 micrometers

7.0 EXPRESSION OF RESULTS

7.1 The effects of the tested material on survival, growth and maturation of the tested population are expressed as percentages of these values for the control population.

7.2 **Survival**

7.2.1 *Survival* is the percentage of survivors in the exposed population relative to the survivors in the control population:

$$\text{Survival} = 100 \times \frac{S_T}{S_C}$$

where S_T = the number of survivors in the test population
S_C = the number of survivors in the control population.

7.2.2 To determine if survival is significantly inhibited by the test material, a chi-square value is calculated:

$$\text{chi-square} = \frac{(S_T - S_C)^2}{S_C}$$

A chi-square value grater than 5 indicates significant lethality.

7.3 Growth

7.3.1 The second toxic effect detected is inhibition of growth, when a significant number of test animals fail to reach the J4 or adult stages. The growth of the test population, relative to controls, can be expressed as:

$$\text{Growth} = 100 \times \frac{(J4_T + A_T)/S_T}{(J4_C + A_C)/S_C}$$

where $J4_T$ and A_T are the number of J4s and adults in the test population, respectively.
$J4_C$ and A_C are the number of J4s and adults in the control population, respectively.

7.3.2 To determine if there is a statistically significantly inhibition (or stimulation) of growth by the test material, a chi-square value is calculated:

$$\text{chi-square} = \frac{((J4_T + A_T)-(J4_C + A_C))^2}{(J4_C + A_C)}$$

A chi-square value grater than 5 indicates a significant effect on growth. If the number of test J4s and adults ($J4_T + A_T$) is greater than the number of control J4s and adults ($J4_C + A_C$) then growth is stimulated, otherwise growth is inhibited.

7.4 Maturation

7.4.1 The third toxic effect detected is inhibition of maturation, when a significant number of test animals that reach the J4 stage fail to reach the adult stage. The completion of the J4-adult moult requires steroid and extensive utilization of genetic information. Specific inhibition of this moult *suggests* toxic effects at the genetic level. Inhibition of the final moult of the test population relative to controls can be expressed as:

$$\text{Maturation} = 100 \times \frac{(A_T)/(J4_T + A_T)}{(A_C)/(J4_C + A_C)}$$

7.4.2 To determine if there is statistically significant inhibition of maturation, a chi-square value is calculated:

$$\text{chi-square} = \frac{(A_T - A_C)^2}{A_C}$$

A chi-square value grater than 5 indicates a significant effect on maturation.

7.5 A summary value, *fitness*, is calculated as the weighted mean of test survival growth, and maturation. relative to the control population. In this calculation, survival carries a weight of 4, growth carries a weight of 2, and maturation carries a weight of 1.

$$\text{Fitness} = 100 \text{ X } \frac{(4 \text{ X } S_T) + (2 \text{ X } G_T) + M_T}{7}$$

where S_T, G_T, and M_T are the survival, growth, and maturation as calculated in 7.2 - 7.4 above.

7.6 The results of a test should be ignored, and the test repeated if any of the following conditions are observed:

(i) Less than 40% of the control population reaches the adult stage.
(ii) More than 20% of the control population dies.
(iii) There is extensive microbial growth in the test cultures that is not present in the control cultures.

8.0 PERSONAL REMARKS

8.1 The primary application of the nematode test is in use as a broad screen for a series of environmental samples or extracts of environmental samples. The test can be used to establish a toxicity ranking of such samples. The test, as described above, is performed at a standard concentration permitting comparisons between samples. It is a good test to include in a battery of tests.

8.2 If the nematode test is utilized for dose-response studies, an LC_{50} value for survival, and separate EC_{50} values for growth and maturation should be calculated. An EC_{50} value for fitness should *not* be calculated, as fitness is not a linear value.

8.3 The biological basis of the test lies in the fact that the development of *Panagrellus* is entrained to the environment; under adverse conditions growth ceases. This is not the case in all nematodes; some will enter a resistant stage, but develop to adults. The toxicity test developed with *Panagrellus* has been shown to work with equal sensitivity in the marine nematodes *Monhystera microphthalma* and *Diplolaimelloides bruciei*.

8.4 It is recommended that the test be performed using one of the standard laboratory strains of *Panagrellus*, as these strains are highly inbred and have little genetic variability. Strain *bq1* is recommended,

8.5 Although the statistical tests provide a means of ascribing differences between the survival and stage distribution of the control and test populations, experience suggests that the following criteria are indicative of toxic conditions:

(i) Survival less than 95%, relative to controls, indicates the presence of materials acting on essential biochemical or physiological processes.
(ii) Growth less than 90%, relative to controls, indicates the presence of materials acting on non-essential biochemical or physiological pathways. The animals are "sick".
(iii) Growth greater than 103%, relative controls, indicates the presence of excess nutrients in the test sample.
(iv) Maturation less than 85%, relative to controls, suggests the presence of materials that perturb of inhibit normal genetic function.

8.6 The nematode test is not a sensitive test for survival, but is quite sensitive for sub-lethal effects.

8.7 The test is easier to perform than it is to describe. The two most difficult aspects are learning to recognize gravid females, and learning the manipulations involved in collecting J2 animals. Both can be learned in one day.

8.8 Several problems may be experience by individuals performing the tests, as they are learning the methodology. The most common problems are a failure of test or control animals to grow. There are two common causes for this:

(i) Lack of cholesterol. *Panagrellus* requires steroids to grow, but cannot synthesize these compounds. Cholesterol provides a source of steroid to the animals.

(ii) Too much fluid in AutoAnalysis cups. If the fluid is too deep, the animals die. Placing only 0.5 mL of fluid in the cups avoids this problem.

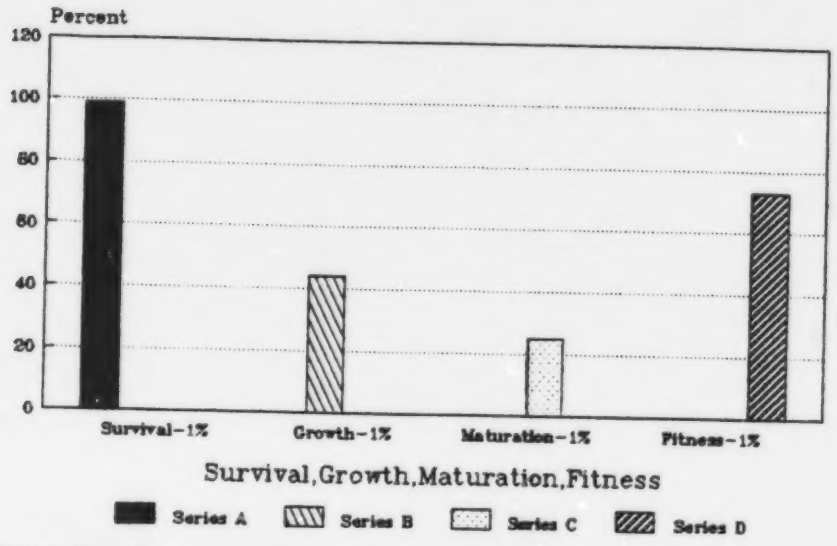
8.9 For tests with methanolic extracts or DMSO, a blank control ("food control") containing M9-Y, and a carrier control containing M9-Y and the carrier material should be set up. Calculations are performed using the carrier control as the reference, although the "food control" is used to ensure that the test meets quality control criteria. When using DMSO, the carrier control should be the same batch of DMSO as used as the carrier.

APPENDIX 2

RESULTS OF NEMATODE TESTS AT ONE PER CENT EFFLUENT CONCENTRATION

Nematode Response 1%

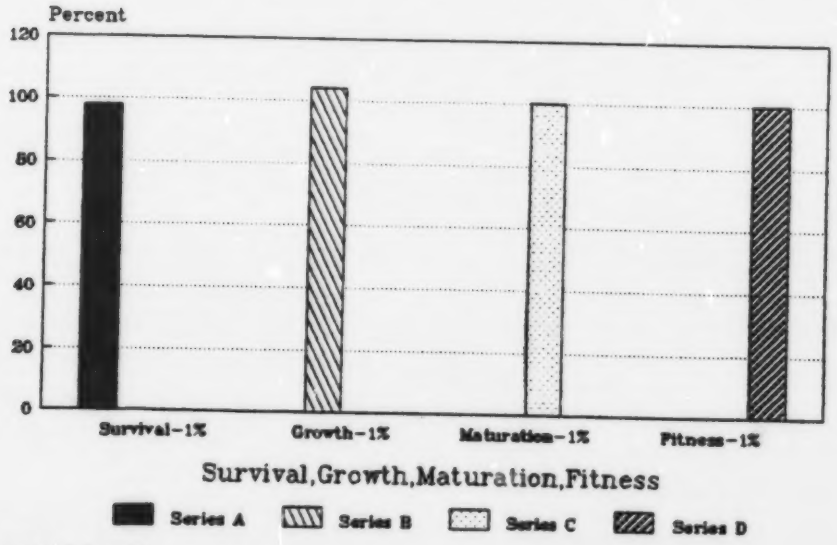
Akzo Chemicals



Grab Samples 17 Aug 90

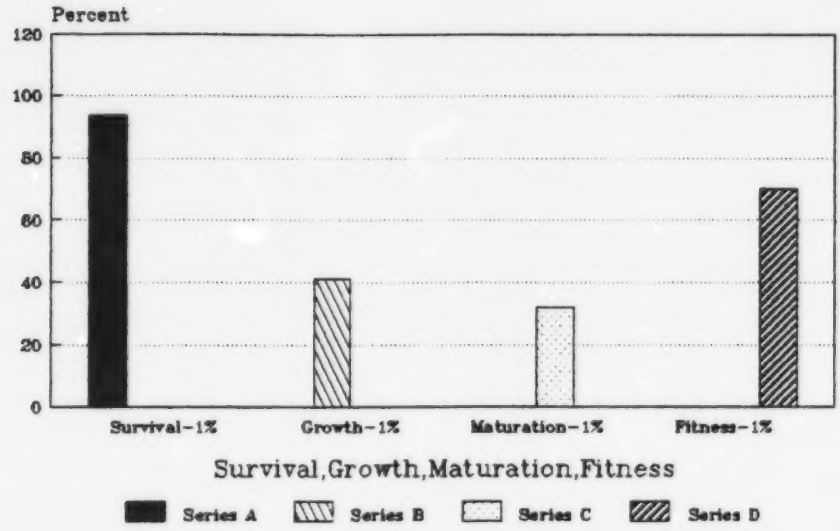
Nematode Response 1%

Akzo Chemicals



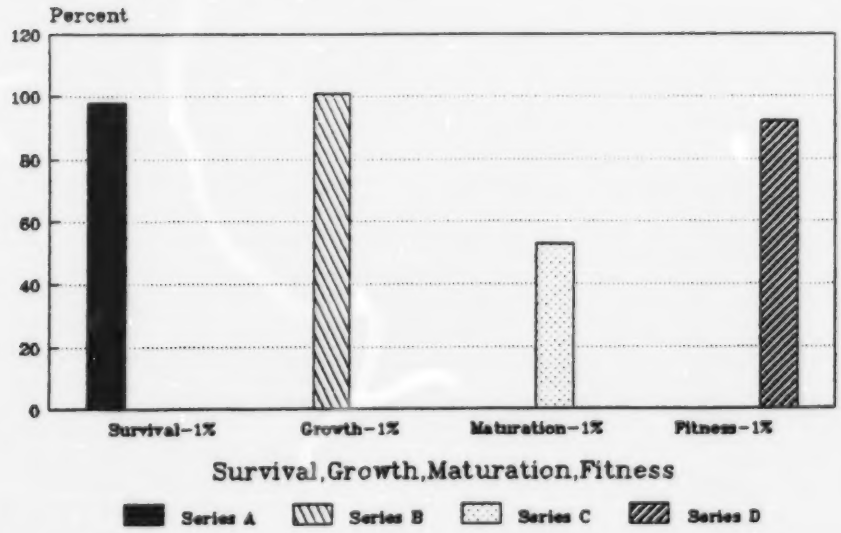
Grab Samples 6 Sept 90

Nematode Response 1%
Saskatoon Chemicals



Grab Samples 17 Aug 90

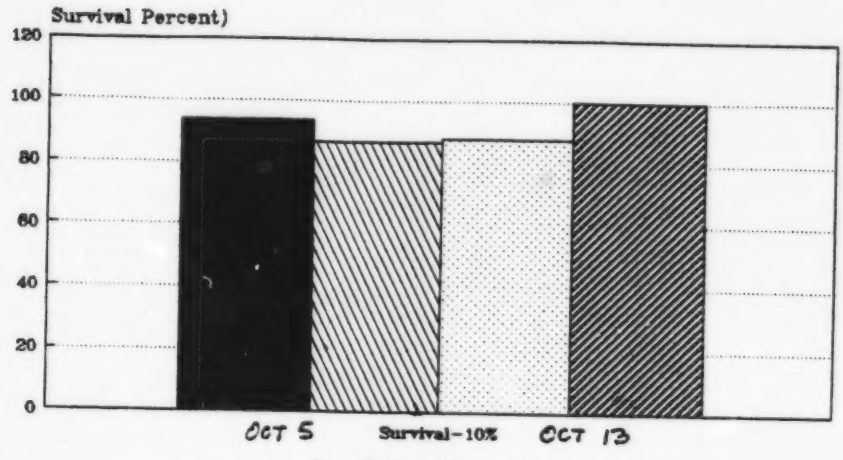
Nematode Response 1%
Saskatoon Chemicals



Grab Samples 6 Sept 90

Nematode Survival %

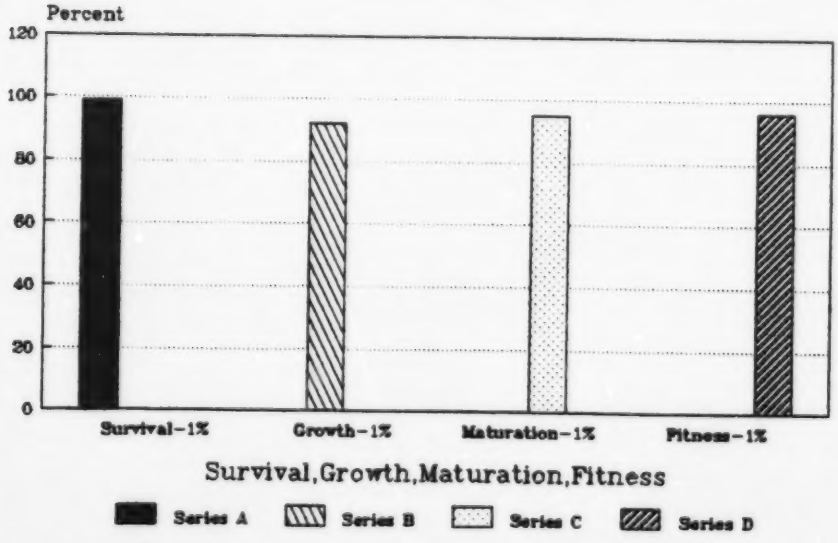
Prince Albert STP



Grab Samples (4)

Nematode Response 1%

Regina STP Chlorinated



Grab Samples 23 Aug 90

APPENDIX 3

SUMMARY OF TOXICITY TEST RESULTS FOR EFFLUENTS

SUMMARY OF TOXICITY TEST RESULTS FOR EFFLUENTS: NUMBER OF SAMPLES CAUSING TOXIC REACTION IN VARIOUS TESTS

Sample	Rainbow Trout	Daphnia	Microtox	Survival Nematode	Growth	Maturation
Effluents						
Akzo Chemicals	1	1	1	1	1	1
	1	1	1	1	0	1
	0	1	1	0	1	1
Saskatoon Chemicals	1	1	1	1	1	1
	1	0	1	0	1	1
	0	0	0	0	1	0
Weyerhaeuser	1	0	0	1	1	1
	0	0	1	1	1	1
	0	0	0	0	0	1
	0	0	0	0	1	1
8th Avenue East Storm Sewer	1	1	1	1	1	1
	1	0	1	0	1	1
	0	0	0	0	0	1
	0	0	0	0	1	1
Boundary Flyash	0	0	0	0	1	0
	0	0	1	0	1	1
Saskatoon Sewage Treatment Plant	1	1	1	1	1	1
	1	1	0	1	1	1
	1	1	1	1	1	0
	1	0	0	1	1	1
	1	0	0	0	0	1
Prince Albert Sewage Treatment Plant	1	1	0	1	1	1
	1	0	1	1	1	1
	1	1	0	1	1	1
	1	1	1	0	1	1
Regina Sewage Treatment Plant	1	0	0	1	1	1
	0	0	0	0	1	1
	1	0	0	0	1	1
	0	0	0	0	1	1
N = 29	18	11	13	14	25	26
Wascana Creek (Receiving Waterbody)	0	0	1	0	0	1
	0	0	0	0	1	1
N=31	18	11	14	14	26	28

0 = no significant toxic response
1 = LC_{50} , EC_{50} at less than or equal to 95% effluent concentration, or for nematodes, $\geq$ 5% inhibited respecting growth or maturation.